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STUDIES ON THE BEHAVIOUR OF
RETICULITERMES SANTONENSIS
(FEYTAUD) IN LABORATORY
COLONIES AND ITS IMPLICATIONS
FOR SOME METHODS OF TERMITE
CONTROL

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S U M M A R Y

STUDIES ON THE BEHAVIOUR OF RETICULITERMES SANTONENSIS (FEYTAUD) IN LABORATORY COLONIES AND ITS IMPLICATIONS FOR SOME METHODS OF TERMITE CONTROL

An investigation of behavioural patterns that form a basis for termite control in the Australasian region was undertaken using laboratory colonies of the subterranean termite Reticulitermes santonensis (Feytaud). The study attempted to build a picture of the behavioural elements of individuals in a colony and based on this, trophallaxis, aggression and cannibalism were investigated in detail.

Preliminary study of food transmission showed that 'workers' played a major role in the distribution of food. It was found, that among factors responsible for release of trophallactic behaviour the presence of 'right odour' between participants was important. It also appeared that the role taken by individuals depended on whether they were hungry or fully fed. Antennal palpation was shown by donors and acceptors alike and this seemed to be excitatory in function.

Introduction of aliens into nests elicited aggression and these aliens were often killed. Factors eliciting aggression were investigated and colony odour was found to be important. Further investigations revealed that development of colony odour was governed by genetical and environmental mechanisms.

Termite response to injury and death was also governed by odour. In the case of injury either the fresh haemolymph from the wound or some component of the haemolymph evoked cannibalism. Necrophagic behaviour was found to be released by fatty acids found in the corpses.

Finally, the response of colonies to nestmates carrying arsenic trioxide was investigated. It was found that living and freshly dead arsenic-carrying nestmates were treated like normal nestmates, resulting in high initial mortality. However, poisoned cadavers soon became repellant and were buried thus preventing further spread of the poison to the rest of the colony. This suggested that complete control of subterranean termites by arsenic trioxide is unlikely to be fully effective, especially in those species which are capable of developing secondary reproductives from survivors and thus rebuilding the community.

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To my wife, Sue, who helped.

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CHAPTER I

CHAPTER 1

INTRODUCTION

1.1. Subterranean Termites and their Control

In those parts of the world viz: the tropics and subtropics, where they are an important part of the fauna, subterranean termites have long been recognized as destructive insect pests. In their natural habitats they are found infesting dead trees, stumps, timber by-products and waste. Such infestations are often regarded as beneficial since the termites return some of the organic and material value of the dead trees, stumps, etc., to the soil and assist in the more rapid destruction of the unusable residue. On the other hand these infestations often constitute a threat to man's agricultural and forest crops, structural timbers and manufactured goods of wood, paper and cloth. The nature and extent of termite damage is fully discussed by Harris (1961, 1965); UNESCO (1962); Greaves and McInnes et al (1967); Hickin (1971); Sands (1973) and others. As a result of these economic losses caused by the termites and the cost of treatment to prevent such losses, considerable research has gone into the biology and control of these creatures. However as Spear (1970) pointed out, the methods of control have not changed much in the last two decades especially in the case of those species, the colonies of which often pose a difficult task to locate.

Harris, Spear, Hickin and Sands (op cit) have all discussed the various measures used for control of termites in the different regions of the world, against the different species of termites found in varying situations. A brief summary of these measures is given below.

(a) In either preventing or eradicating an active infestation in buildings and other timber structures, primary consideration has been given to planning and constructional practices and to the use of material either resistant or made resistant to termites by the use of chemical preservatives. This has been further supplemented by the application of persistent chlorinated hydrocarbon insecticides in and around the area where the buildings or timber structures are sited. To terminate an existing infestation the same basic principal is used except that the structures in which the termites are found are drilled with holes 18-24 inches apart and the insecticides pumped through these holes into the galleries and runways of the pest. In the S.E. Asian and Australian region toxic dusts (mostly Arsenic trioxide) are used more often than chlorinated hydrocarbons.

(b) In the control of termites in crops, wherever mound-inhabiting species are found to attack, the mounds are poisoned thus bringing about the eradication of the colony. Where attack of the crops is brought about, not by mound building species, but by other species whose habits are more cryptic, control is made more difficult because of the inability of the controller to get the poison to the target (the whole colony). Control is therefore, more often attem-

pted by the application of a wide range of insecticides including arsenicals to the soil in and around the area of infestation or by local application to the collar of the plant, to potting mixtures or by seed dressing. was, of course,

Because of the cryptic nature of the subterranean ^{lined} termites, most of the above control measures have one broad objective, viz: the transmission of enough poison to all the members of the colony, including the reproductives, that are causing the damage. To achieve this objective, termite controllers rely on some aspects of the social behaviour of termites especially those elements which involve mutual grooming, mutual exchange of food and the disposal of injured, dying and dead nestmates. In normal healthy colonies nestmates are constantly grooming each other, exchanging food and cannibalizing the dying and the dead - and as such it is believed that similar interaction also takes place ^{lined} with individuals either returning with poison on or within their bodies or those killed by the poisons. Immediate cessation of termite activity on being subjected to such ^{on} poisoning seems to indicate that a certain amount of success was being achieved; however reinfestations of treated areas seemed also to cast doubts on the reliability and permanence of such treatments, even though the chemicals used were of the persistent type. This raised some questions with regard to the control measures used, viz:

- (1) The factors that influence trophallaxis,
- (2) The factors that influence cannibalism, and
- (3) The interaction (if any) between healthy and poisoned individuals in a colony.

This study was started, in order to obtain tenable answers to the above and other questions about the behaviour of individual termites. Since termites do not occur naturally in Great Britain, the present study was, of necessity, confined to laboratory colonies. This, of course, limited the choice of species available to a few, maintained by the various laboratories in the country. Thus in choosing the experimental termite the following criteria were considered:

- (1) The termite has to be subterranean.
- (2) It should be "available" in England.
- (3) It should have a high reproductive potential, and
- (4) It should be suitable for laboratory culturing.

Reticulitermes santonensis (Feytaud) fulfilled the above criteria and it was cultured at the Forest Products Laboratory, Princes Risborough. Two small colonies were obtained from F.P.R.L. and these formed the source of all subsequent sub-cultures used in the study. A small supply was also obtained from the Centre for Overseas Pest Research, London for use in some experiments (Chapter 5).

1.2. The Study of Termite Behaviour

The spectacular mounds of termites, their building of these, the foraging columns and the nuptial swarms of these insects, have attracted the attention of many naturalists from early times. A detailed account of termite societies was given to the Royal Society of London as early as 1781 by Smeathman (Stuart, 1969). Similar observations were made by Beaumont (1889-1890) on the behaviour of termites of Panama and Grassi and Sandias (1896-1897) recorded the behaviour of the European termite based on some simple experiments. These and other observations by early zoologists have all become part and parcel of the vast anecdotal literature on the behaviour of termites. While many of the elaborate hypotheses suggested by the early workers have been instrumental in provoking further investigations, the information provided by them, regrettably, is of limited use to the contemporary ethologist because of its incidental nature.

Many modern studies have been concerned with the behaviour of the whole colonies rather than individuals, e.g. Andrews (1911), Fuller (1915), Emerson (1939), Grasse (1952) and Weesner (1960) among others. Furthermore there has been a strong tendency to adopt a behavioural-cum-physiological approach rather than a purely behavioural one. This former approach which has produced some very interesting results, is instanced in the works of Richard (1950), Abushama (1966), Howse (1964a, 1964b, 1965) and Stuart (1961, 1964, 1967). Alternatively very few studies have been undertaken on the patterns of behaviour of individual

termites although Stuart (1969) has suggested that 'more work should be carried out on the behaviour of single termites as individual organisms'. The few studies that have been carried out on individuals have been confined to areas like pre-flight, dealation, pairing, nest founding and copulatory behaviour (Weesner, 1960; Noirot, 1969; Harris and Sands, 1965; Stuart, 1969; Nutting, 1969 and Howse, 1970). Generally there has been a reluctance to follow the behaviour of one individual for any length of time.

Since colony integration and colony function can only be understood in terms of the response patterns of the individual, the need remains for a fairly intensive, but comprehensive investigation of the whole behavioural repertoire of the termite. Only then will it be possible to see how particular response patterns, e.g. inter-caste and inter-stage responses, fit into the overall pattern of behaviour of the individual.

This study attempts to make such an investigation. An analysis has been made of the responses, many of them social, which may be shown by the individual termite. Three major lines of enquiry were pursued. These were:

- (1) trophallaxis,
- (2) aggression, and
- (3) cannibalism.

Since the aim of the study was not only to contribute to an understanding of the behaviour of termites but also to throw some light on the possible effects of a toxicant on such

behaviour, a fourth line of enquiry investigated was the effects of Arsenic trioxide poisoning on the behaviour of these insects.

While the study was in the main confined to analysing behavioural patterns, some attempts were made to understand the physiological basis for such behaviour. This aspect was, however, looked into very briefly, due to the limitation of time available for the study and also due to the author's limited competence in the field of physiology.

TERMS AND METHODS

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CHAPTER II

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CHAPTER II

MATERIALS AND METHODS2.1. The distribution and taxonomic position of *Reticulitermes santonensis* Feytaud.

Subterranean termites of the genus Reticulitermes are found in the temperate regions of the world. In Europe - West of Roumania - three species are known to occur. The first, R. lucifugus Rossi is found from the islands of Madeira in the west, to the borders of Turkey in the east (Harris, 1961). The second, R. flavipes Kollar, a north American import, was first discovered in the green house of a palace near Vienna in the nineteenth century and is found today in some areas of Hallein, Austria and Hamburg, Germany (Hickin, 1971). The third species, which is used in the present study, is R. santonensis Feytaud (= R. lucifugus var. santonensis = R. flavipes santonensis). It is found in the provinces of Charentes, Vendee, Loire Atlantique, Deus Servs, Ile d'Oleron and in Paris (Mathieu, 1959, 1962).

Since its 'discovery', in 1925 by Feytaud, the exact taxonomic position of the 'termite de Saintonge' has been a source of confusion among taxonomists. In his examination of various populations of R. lucifugus, Feytaud noticed striking differences in the behaviour of some. The variant, Feytaud discovered, had greater fecundity, increased resistance to drought, attacked plants and woodwork with greater vigour and finally showed differences in their intestinal fauna. Feytaud (1948) thus concluded that the 'saintonge

termite' was not R. lucifugus but an introduced form of R. flavipes and proposed the sub-specific name santonensis. Williams (personal communication) based on his own investigations agrees with Feytaud and believes that the 'saintonge termite' is a variant of R. flavipes. But Lash (1952), Emerson (according to Buchli, 1958) and Buchli (1958) believe the variant to be a race or subspecies of R. lucifugus and not R. flavipes. Buchli (op cit) however concedes that in its biology the santonensis termite shows greater resemblance to R. flavipes rather than R. lucifugus. Based on the morphological examination of just soldiers alone Thurlich (1960) expresses the view that santonensis is a race of R. lucifugus and not of R. flavipes.

Based on numerous morphological, bionomical, ecological and behavioural investigations in the field and the laboratory Becker (1961, 1965a, 1965b and 1971) came to the conclusion that the 'saintonge termite' is probably a cross-breed between R. flavipes and R. lucifugus. Thus he proposed that the termite be called R. santonensis. Some of the reasons for Becker's conclusions are:

- (a) In its morphology, temperature dependance and gallery building behaviour the santonensis termite resembles R. flavipes.
- (b) In its humidity requirements the saintonge termite resembles R. lucifugus.
- (c) The saintonge termite has a flagellate fauna similar to that of R. flavipes and R. lucifugus.

- (d) The saintongetermine exhibits a daily rhythm, vigour in activity and resistance to climatic factors quite unlike R. flavipes and R. Lucifugus.

Becker has not established beyond doubt that the 'saintonge termite' is a crossbreed of R. flavipes and R. lucifugus and neither has there been definitive proof to establish it as a variant of one of the two species above. In view of this lack of evidence, it is proposed in this study, to refer to the 'saintonge termite' as Reticulitermes santonensis especially taking into account the fact that it seems to have less in common with R. lucifugus than R. flavipes.

2.2. The biology of *Reticulitermes santonensis* Feytaud.

As pointed out earlier, *R. santonensis* is a subterranean creature and is known to attack all kinds of wood both living and dead and is considered something of a pest in its natural habitat (Buchli, 1958). Almost all the information that is known of the biology of *R. santonensis* has been reported by Buchli (1950, 1958 and 1961).

R. santonensis is capable of founding new colonies without the accompanying swarming flights. Whether colonies are founded by swarming sexuals or neotenic individuals they (i.e. the sexuals or neotenic individuals) go through a protracted courtship. On pairing, the termites find a nesting site, normally dead trees, branches, stumps, posts and other materials into which they excavate a chamber. This is followed by copulation. Very little is known of the factors involved in the selection of the nesting sites; Nutting (1969) suggested that in wood dwellers at least the alates might orient visually.

Within a week of copulation the queen begins to lay eggs and in a month she would have produced 10-20 eggs. At 25°C the eggs hatch in 20 to 25 days. The larvae of the first two stages are all alike and remain undifferentiated. The developmental period of the first instar ranges from seven to eight days and that of the second from ten to eleven days. In small primary colonies all these become "workers" at stage three, but Buchli records that in large natural colonies they become nymphs showing small wing pads.

"Workers" differentiating in stage three and remaining as workers thereafter may moult regularly through stage nine. This may take about 160 days. Moults do occur after the ninth in some cases, but these produce very little change in form.

However "workers" can become sexuals, especially at stages five and six, and in fact they (i.e. the 'workers') can redifferentiate into all castes in a big colony. Hence in a strict sense no fixed adult form to be called "worker" exist in this species.

Pseudergates or 'false workers' can come from stages three to six by regressing in character, or from larvae by stationary moults which fix their characters between those of nymph and worker. Beyond stage five, pseudergates, by redifferentiating can form either reproductive neotenics or soldiers.

Soldiers can develop from workers, pseudergates or nymphs of stages five to seven (or stage four in primary colonies). The individual destined to be a soldier goes through a pre-soldier or a white soldier stage. Both sexes may produce soldiers.

Neotenic reproductives can develop from 'workers' of stage four or later, from pseudergates of stage five or later or from nymphs of stage six or later.

In primary colonies, even though the functional imagoes are present, development of neotenics is not suppressed, as

long as the nutritional equilibrium of the group is favourable. However when the nutritional equilibrium is unfavourable the development of further sexuals as well as soldiers may be suppressed or delayed in appearance.

2.3. The question of caste differentiation in lower termites.

Discussion on the question of caste differentiation in the lower termites started with a paper by Grassi and Sandias (see Miller, 1969). Miller further stated that this was followed by observations or investigations by Lespes (1856); Mueller (1873); Holmgren (1912); Heath (1927); Imms (1919); Jucci (1924); Snyder (1925); Thompson (1922); Kalshoven (1930); Weyer (1930) and others. In recent years this problem has been further tackled by Castle (1934), Light (1944), Hill (1925), Pickens (1932) and more recently by Lüscher et al (1952-1969).

The works of these authors resulted in the formation of two schools of thought. The first school 'the extrinsic school' (Grassi and Sandias (1893-94), Escherich (1909) and Holmgren (1912)) reasoned that all termites have equal potential upon hatching and that their caste is fixed by environmental factors acting on them later. These extrinsic factors were believed to include differential nutrition or physiologically active exudates that originate from nest mates.

The opposing 'intrinsic school' as hypothesized by Imms (1919) suggested that castes are genetical segregants. Miller (1969) reported that supporting evidence for this was provided by Thompson who found in her investigations two egg sizes and possibly correlated with these two kinds of larvae those with small heads, large brains and large gonads destined to be reproductive forms and those with larger heads, smaller brains and smaller gonads destined to

become workers or soldiers. Such dichotomy has not been detected in any of the later studies.

The above theories have now been replaced by the concept of inhibitory substances (more akin to the extrinsic theory) which was first suggested by Pickens (1932) and for which some experimental evidence was provided by Castle (1934) and Light (1944). More evidence of this has been provided by the work of Lüscher and his group on Kalotermes. This theory states that the members of the reproductive castes give off pheromones or ectohormones, containing inhibitory substances which are transmitted to nymphs through mutual feeding and grooming throughout the colony. In this way the reproductives and soldiers exert an inhibitory influence on the development of like forms. When, because of an increase in population due to colony growth or because of the loss or reduction of reproductives or soldiers, some undifferentiated nymphs fall beyond this inhibitory influence, they develop into additional members of these castes.

Buchli (1958) did not believe that there was evidence suggesting an inhibitory hormone as a major directive factor in the determination of castes in R. santonensis. He however concurred with Lüscher that within each larval or nymphal stadium there is a critical phase or 'reactivity' period when the individual is susceptible to extrinsic influences. He considered that the first two larval stages were strongly affected by nutritive conditions and nymphs and workers by "perturbations" of the social equilibrium of the group.

2.4. Laboratory culture methods

The termites used in the present study were obtained through Miss J. Taylor of the Forest Products Research Laboratory. The original colonies were obtained from the Saintes region of France.

Because of the extensive use of termites as laboratory test insects, various authors (Becker, 1969; Gay et al, 1955; Hrdy. I., 1964) have developed cultural methods for different species. To a great extent culturing techniques used in this study are a minor modification of those used by Becker (1969).

Containers:

Stock colonies were maintained in fibre glass tanks measuring 40 x 33 x 30 c.c.m. (cubic capacity app. 45 litres). The containers were placed on raised platforms in the middle of metal trays measuring 80 x 40 x 4 cm. These trays were then filled with water. The top of each fibreglass tank was kept covered by a loosely placed glass plate; the bottom of each tank was perforated with tiny (3mm) holes and these holes were covered with a fine mesh of wire gauze. This allowed for the drainage of excess water from the substrate and at the same time prevented the termites from crawling out. The lid covering the mouth of the tank was sufficiently loose to maintain adequate aeration whilst stopping termites from crawling out or alates from flying out (Plate 2.1). It is worthwhile to note that alates have not been obtained in the Aston laboratory cultures nor at the Forest Products Research laboratory in

Plate 2.1. Fibre glass containers with water barriers for R. santonensis cultures.



this country (J. Taylor, personal communication).

Substrate:

Since R. santonensis is a subterranean species, the fibreglass tanks are quarter filled with clean, sterilized soil of uniform particle size. Humus when present in the soil was left in it. No consideration was given to pH or other chemical characteristics of the soil. Though the cultures in this laboratory were maintained only on soil other workers have used sand, peat and sawdust (Lund, 1967).

Food:

Subterranean termites are capable of living on any material containing cellulose. Becker (1969) considered that wood with low density is normally preferred to wood with high density. He also considered that spring wood is preferred to summer wood. However the question of termite wood preference is still very debatable. While factors such as moisture content, sap or heartwood presence, cellulose/lignin ratio, extractives, etc., do provide some answers to termite wood preference they do not answer all. Lund (1967) reported that genetic factors might have an effect and Esenther et al (1961) stated that decay of wood by Basidiomycetes of the brown rot type influenced termite wood preference.

Wood specimens used in this laboratory for termite cultures were either Ilomba (Pycnanthus angolensis) or sitka spruce (Picea sitchensis K). Throughout the length of this study colonies maintained on Ilomba were maintained on Ilomba and those on spruce on spruce. Though Becker (1965b)

advised the use of wood blocks with 3-10% weight loss due to attack by brown-rot, no such measures were specially taken in these cultures. The wood blocks which were oven dried were left in the tanks where they became infected with fungi and bacteria.

Temperature:

Colonies in the fibre glass tanks were maintained in a room the temperatures of which varied between 21°C-25°C. Becker (1969) considered that a temperature between 26°C and 28°C was most favourable for breeding and testing termites.

Humidity:

Most termites require relative humidities in the range of 95 to 98% within their normal temperature range (Lund, 1967). In the culture room of this laboratory an adequately high humidity was maintained by using a domestic humidifier which evaporated approximately one pint of water every six hours, which gave a R.H. between 90-95%.

Within the tanks a high moisture content of the substrate and food was maintained by adding up to about 15 or 20% distilled water periodically.

Aeration:

Termites can tolerate air with a relatively high CO₂ content (Grasse, 1949; Lüscher, 1961) and also extensive aeration can be detrimental as it leads to desiccation of the colony unless the ambient humidity is maintained at a

high level. The loosely placed glass plates which covered the tanks in this study provided adequate aeration while preventing excessive evaporation.

2.5. The observation plates

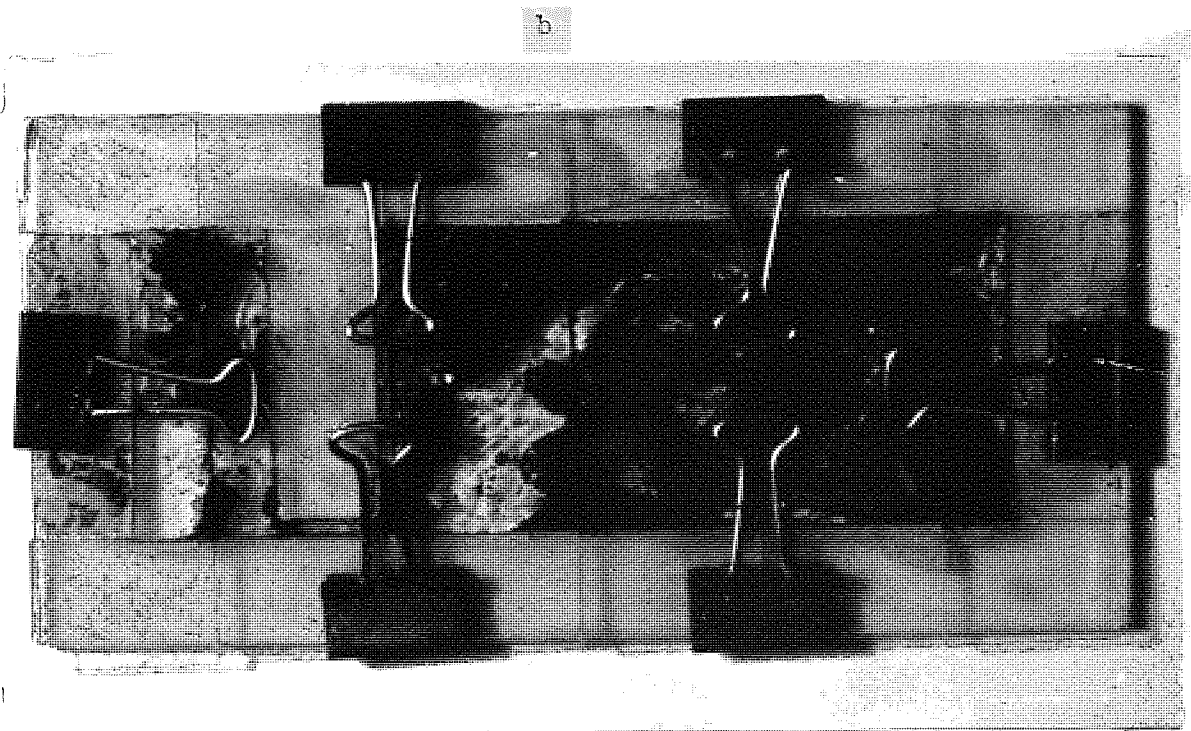
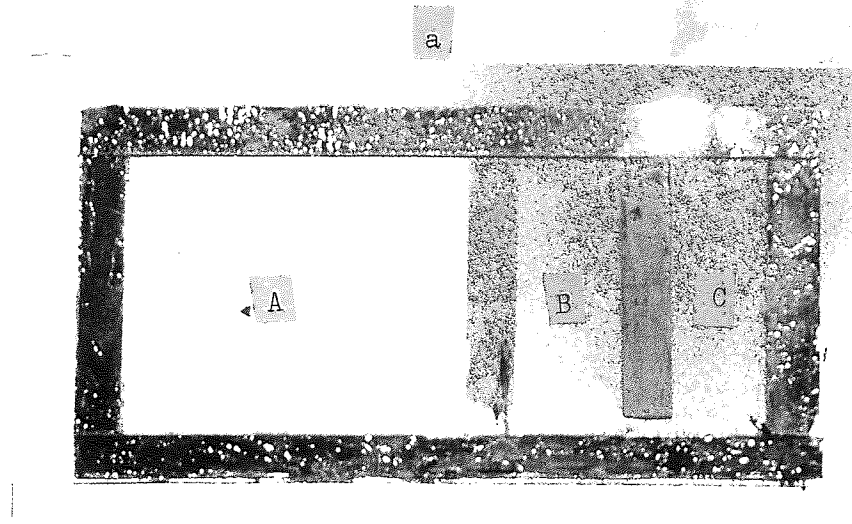
For a greater part of this study the termites had to be kept under continuous observation. This necessitated the use of breeding methods that would facilitate observation. Such methods have been used and found to be satisfactory for ethological studies of other social insects like ants and bees (Wilson, 1962; Free, 1955; Butler, 1954). Though Skaife (1955) had used glass plates for studying higher termites, he found them to be unsatisfactory. Most other workers have used tubes, jars and petri dishes (Becker, 1969) as containers for termites. These allowed observations for a limited period of time after which observations were completely obscured, by the deposition of soil, food and fecal material, by the termites.

Glass plates were first used by Adamson (1941) when studying several Neotropical spp. His glass plates were later modified by Lüscher (1949). The method used in this study is essentially similar to Lüscher's but with minor modification.

Plate 22a is a photograph of the type of observation nest used in this study. Narrow glass strips 3mm thick and measuring 15 x 1.5cm and 4.5 x 1.5cm were glued to the perimeter of a 3mm thick glass plate measuring 15 x 4.5cm, giving the nest a depth of 3mm. Dependent on the type of experiment, the chamber of the nest was either subdivided into two or three inter-communicating sub chambers using glass strips as barriers. A 3mm gap was left in the barrier which was sealed with glass wool whenever the chambers required isolation from each other. Since the termites were fed either

Plate 2.2. Glass plate termitaries for continuous observation.

- (a) Empty plate showing the different chambers
- (b) Plate with termites and wood flour within.



on Ilomba or spruce, decayed wood of the appropriate species was milled in a hammer mill to pass through a 1mm mesh sieve. This 'wood flour' was then used to fill one of the sub-chambers of the nest (normally chamber A in Pl.2.2.), where it acted both as food and substrate for the termites. The nest was then ready to receive the termites. Termites were transferred from culture tanks to the glass plates by methods described by Gay et al (1955). Healthy and active termites were normally left in Chamber 'B' of the glass plate nest, from where they normally found their way into the food/substrate chamber. Each nest normally contained 194 'workers' (3rd, 4th and 5th stage), 4 soldiers and 2 nymphs (a male and a female). The nymphs generally developed into supplementary reproductives within a short period. After the introduction of the termites into the nest, the chambers were covered with five 74 x 25mm microscope slides. These were fixed to the nest by "Handgrip" fold back spring clips. The cracks between the slides provided enough aeration for the termites. Distilled water was also introduced to the substrate/food through these cracks, thus enabling moistening of food and the relative humidity within the chamber.

Once inside the nest the termite workers bore runways through the tightly packed wood flour in sub-chamber A. In a short time they established inter-connecting runways, within the wood, and all activities of the termites were then easily continuously observed.

In order to replenish the colony with food when it ran out, glass slides covering the food chamber were gently

removed. This caused the termites to move into Chambers B and C; when all had moved into these chambers more wood flour was packed into Chamber A, which was then moistened and recovered with the glass slides.

The glass plate observation nests have worked well throughout the course of this study. The termites settled well in the plates and eggs have been laid continuously and larvae brought up.

The nest described above has allowed continuous observation of the termites for hours under the microscope. Because R. santonensis is a wood dwelling species the use of only wood flour without soil or any other material has not prevented them from indulging in their building and other social activities.

2.6. Recording of behaviour

The termites were allowed to settle into their plate³ nests before observations on their behaviour were commenced. This normally took place at least a fortnight after the introduction into the glass plates.

All observations were carried out under a binocular microscope with a zoom lens using magnifications between X10 and X20. Illumination during observation was kept to the minimum by cutting out all other wave lengths except red (600-700m^u). This was done by passing the light through a Kodak Wratten filter no. 25. Skaife (1955) found that termites 'preferred' red light to others. This was found to be so in this study as well. There was a considerable amount of agitation when the colonies were subjected to unfiltered light and this (agitation) was almost nil under red light. A heat filter was also used to reduce radiant heat as much as possible during observation.

Before recording of behaviour was commenced a basic repertoire of behaviour was first established. This was carried out on the basis of functions such as grooming, running, feeding, etc. Each behaviour was assigned a symbol, and in recording a sequence of behaviour, the normal method was to record a spoken commentary on a tape recorder. The commentary recorded described the type of response and from this a written record using the symbols was made on a tabulated format. A minute by minute record was made for analysis.

Since it was the intention of the study to observe the individual behaviour of the termites, it became necessary to mark them. This was done by using black cellulose paint. Using a fine wire a spot was made on the dorsal side of the head capsule. As long as the spots were minute enough they did not attract undue attention from the rest of the colony.

Where techniques different from the above were used they are described in the appropriate chapter.

CHAPTER III

CHAPTER III

THE BEHAVIOURAL REPERTOIRE OF
RETICULITERMES SANTONENSIS FEYTAUD

3.1. Introduction

An ethogram (a complete description of behaviour) of a termite does not seem to have been undertaken before. Nel et al (1969) produced a very brief description of the activity of Hodotermes mossambicus (Hagen). They divided the activity of that termite into the following components:-

- (a) "Stationary": When the individual did not alter its position although it moved its legs and antenna.
- (b) "Moving": The individual walked or ran about but did nothing else.
- (c) "Grooming in": The individual was 'groomed' by other termites. This consisted of "licking" all aspects of the abdomen, the ventral side of the head, the legs and antenna.
- (d) "Grooming out": The individual 'groomed' another termite.
- (e) "Trophallaxis in": The individual and another termite stood head on. Their mouth parts and antennae touched and liquid was transferred from the other termite to the individual under observation.
- (f) "Trophallaxis out": As in (e) except the liquid was transferred from the individual to another termite.
- (g) "Feeding on": Bits of food (grass) were carried about or nibbled by the individual.
- (h) "Handling Faeces": Faecal material often in the form of pellets was carried in the mouth.
- (i) "Handling Eggs": Eggs were picked up in the mouth parts and carried or moved.

- (j) "Carried": The individual was picked up by another termite and carried about.
- (k) "Carrying": The individual picked up another termite and carried it about.
- (l) "Anal Licking In": The individual collected the faeces of another termite as the faeces was passed.
- (m) "Anal Licking Out": The individual remained stationery while another termite collected its faeces as it was passed.

As the above description was found to be inadequate for the present study, it was decided to make an attempt to (a) classify and (b) characterize the different sorts of behaviour an individual termite may show. The study was carried out on four different colonies. Each colony was made up of at least two hundred individuals composed of a pair of neotenic reproductives, four soldiers and one hundred and ninety four 'workers' (third, fourth and fifth stage). The colonies were set up as described in Chapter 2 and left undisturbed for about a month, when observations began. All colonies had started reproducing by this time and there were many eggs and larvae about. Each observation period lasted thirty minutes and was confined to a single individual of one of the following castes or stages:-

- (a) Larvae: 1st stage: newly hatched larva
2nd stage: larvae that had undergone the first moult.
- (b) Workers: Adult workers normally the third, fourth or fifth stage.
- (c) Soldiers: Mature pigmented individuals with well developed mandibles.
- (d) Replacement Reproductives: Normally pseudergates of the sixth stage that develop into reproductives by redifferentiating.

Plate 3.1. Workers of R. santonensis in a typically alert position.



3.2. Reticulitermes santonensis Feytaud.

Plate 3.1 is a photograph of a worker of R. santonensis in a typically alert position.

All figures and descriptions in this chapter refer to worker termites unless otherwise stated.

3.2.1. Stationary and Moving Termites.

In plate nests in the laboratory, worker termites are seldom inactive though there are times when they remain stationary for a long period. On the other hand replacement reproductives, soldiers and larvae remain stationary for long periods. Termites were considered to be stationary when they remained inactive without making any movement of the body for more than fifteen seconds; and when the individual extended its neck or body or rotated its head, walked or ran it was considered as terminating its stationary phase. There is, however, no rigid distinction between a totally stationary or a totally moving animal. Intermediate postures between the two extremes are frequent.

When the termite is moving it performs a number of activities. These activities are either carried out;

- (a) alone by itself, or
- (b) with another individual or individuals.

Heyde (1924) whilst describing the behaviour of Companatus, Myrmica, Lasius and Formica described (a) as "individual instincts" and (b) as "social instincts". Wallis (1962) on the other hand described (a) as "behaviour not involving another

individual" and (b) as "behaviour involving another individual". In this study it is proposed that (a) be referred to as "Individual Behaviour" and (b) as "Social Behaviour", since it was felt that the term 'instinct' was inefficient to explain some of the complex behaviour of these insects.

3.3. Individual Behaviour

3.3.1. Walking/Running

These patterns were essentially the same except for the variation in speed and posture adopted. The antennae were normally held at least 10° above the horizontal, outstretched from the body and in a constant slow sweeping motion. However, there were times, especially when the animal was following a trail, when the head pointed downwards and the antennae swept the substrate in a rotary fashion.

There was no variation from the above description in the execution of this activity by the other castes.

3.3.2. Feeding/Drinking

In the colonies under observation only the workers were involved in actual feeding and drinking activities. The worker normally pulled small fibres from the wood flour substrate and masticated them until the wood was completely engulfed. Most times only small particles were scavenged from the surface and in these operations the maxillae and labium were extensively used. The antennal tips were directed downwards and often rested on the surface that was fed on.

Drinking was seldom observed in the plate colonies, but on the few occasions it was performed the termite applied its mouth parts firmly to the surface from which it was drinking and the fluid was imbibed by lapping movements of the labium.

Replacement reproductives, soldiers and larvae, did not feed independantly and were dependant on stomodeal and

proctodeal material from the independant feeders (see Chapter IV).

3.3.3. Carrying

Again only workers were observed performing this activity. In observation plates the only particles carried were wood fibres used in sealing surfaces or smoothing run-ways. The particles were lifted from the substrate and were carried in the mandibles.

The other castes under observation did not show any carrying behaviour.

3.3.4. Building

There was little stimulus in observation plates for the complete performance of building activity, but individual termites did go through the sequence of behaviour associated with building activity. This began with exploratory behaviour during which the worker examined gaps and crevices in the glass plates with its antennae. This was repeated a number of times. Following this the worker searched the chamber for a "suitable" particle which it grasped between its mandibles and returned to the point previously explored. It then either (a) inserted the particle in the crevice and extruded a drop of salivary "cement", or (b) it first brought the tip of its abdomen to the crevice and expelled into it a drop of liquid "cement". Thereafter it turned around and "fixed" the particle in position. There were times when the termite did not collect a particle to seal a gap but merely used its stomodeal or proctodeal exudate to do the job. Howse (1970) discusses nest

building behaviour in Zootermopsis nevadensis (Hagen) in detail and stated that a "sensory tape" or comparison system was used where a number of components were involved in nest building and that each component of the behaviour had a sensory feed back mechanism, i.e. 'the termite must receive specific sensory information indicating that one component (of the behaviour pattern) was completed before another could continue. Once one component had been completed, it never again occurred in the same sequence'.

Various hypotheses to explain the methods of nest construction and the stimulus necessary for it have been put forward and discussed by Grasse (1959), Harris and Sands (1965), Howse (1966) and Stuart (1967).

Only workers were involved in the building behaviour observed in this study.

3.3.5. Grooming

The act of grooming may serve three major functions, viz.

- (a) Removing unwanted or foreign particles, etc., from the body (Wilson, 1971).
- (b) The coating of the body with a sticky saliva to protect the surface from excessive or lack of moisture (Wilson, 1971), and
- (c) Wheeler (1910) suggested that the growth of harmful moulds and bacteria are prevented through grooming.

Cleaning movements of termites have never been described in any detail, though on the other hand those of other social insects (ants and bees) have been observed and recorded by numerous workers. This might be due to the fact that the termite does not perform spectacular cleaning movements on itself as do so many ants. However termites do perform a variety of cleaning movements as described below.

In its essentials, the cleaning behaviour of all castes seems to be similar to that of the worker and the description below is that of a worker.

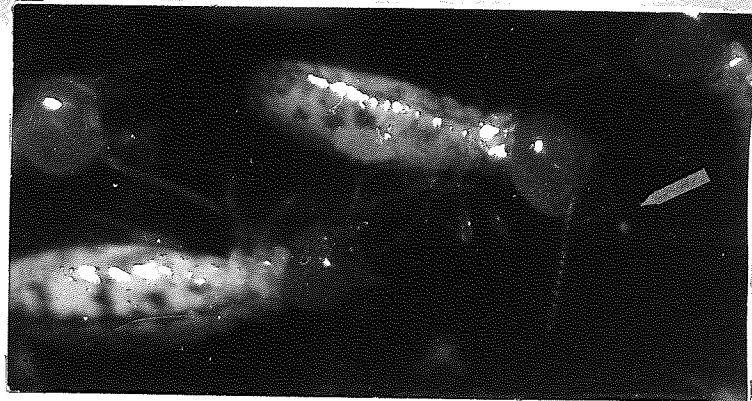
Grooming, insofar as they are primarily cleaning movements, may be best classified by the parts of the body that are cleaned. Three main regions were distinguished

Region 1: Head Grooming

Areas of the head that were cleaned by the termite itself included the mouth parts, the antennae, the anterior genal and the ventro-lateral areas of the head capsule.

(a) Plate 3.2 shows a worker cleaning its mandibular and labral mouth parts. This it did by remaining stationary, raising its head slightly, and holding its antennae in a wide angle from the head capsule. The maxillary palps were then run over the labrum and mandibles from top downwards thus sweeping particles sticking on the surface into the stomodeal opening. The mandibles were also opened and closed in a masticatory motion as the palps were run down. The cleaning stops just below the anteclypeus.

Plate 3.2. Self grooming - cleaning of mandibles.



(b) The antennae were normally cleaned alternately. A typical antennal cleaning was carried out as follows:

The animal remained stationary as in (a). There was a quick rotatory movement (the direction of the rotation was dependant on the antenna that was cleaned) of the head. Then the antenna was brought down and around with its base in between the maxillary and labial palps. The antenna was then slowly drawn out article by article through the groove formed by the palps. Any foreign particle sticking on the antenna was thus removed, whilst the antenna was also coated with the salivary secretion.

Often only one antenna was cleaned at any one time. This may be repeated twice or thrice. Cleaning ceased after this. Antennal cleaning was the most frequent self grooming act performed in laboratory colonies.

(c) In the grooming of the genal and latero-ventral regions of the head the termite remained stationary and the head was inclined towards the side that was to be cleaned. Then the foreleg of that particular side was used to brush down the region - the protrusions of the tarsi acted as brushes. While this cleaning was done the termite rested on five legs. When the ventral side of the head was cleaned the head was normally raised and when the genal area was cleaned the head was lowered and bent inwards.

Region 2: Thoracic Region

Self cleaning of the thoracic region was limited to the legs only. The animal remained stationary and bent its head inwards towards the side of the limb that was to be cleaned. The leg was then brought forward and run through mouth parts. Cleaning was confined to those regions of the leg that were accessible viz. the tibia, tarsi and claws. Of the three pairs of legs, the forelegs were cleaned most frequently.

Region 3: Abdominal Region

Cleaning of the abdominal region was also confined to limited areas such as the lateral and ventral regions. In cleaning the abdomen the worker used its hind legs (the spined tibial region). It rested on five legs and used its sixth (either side) to brush down by a 'one stroke movement'. This was occasionally repeated. Cleaning was then broken off. Particles that stuck on to the legs were later removed during cleaning by other termites.

All these cleaning patterns were identical in all instars and castes beyond the second stage larva. First and second stage larvae seldom indulged in self grooming.

3.4. Social Behaviour

3.4.1. Examining

Wallis (1962) defined 'examination' in ants as 'orientation towards an individual, accompanied by a stretching and orientation of the antennae towards that individual'.

In termites it is a similar movement except that the animal carrying out the examination touched the individual being examined. Antennal examination was often accompanied by 'feeling' the other individual with the tips of the maxillary palps.

By examining another termite information is presumably obtained through the chemoreceptors in the antennae and maxillary palps. It is possible that tactile information is gained as well since the antennae and maxillary palps touch and move over the surface of the examined termite.

Orientation of the antennae, etc., may accompany other behaviour such as grooming, threatening, attacking, or on exchanging food with another termite. It was classified as examining, only when the other elements were absent.

3.4.2. Grooming

Besides cleaning, grooming of another individual presumably fulfils two major functions, viz:

- (a) The coating of the other termite with saliva.

Plate 3.3. Grooming others

- (a) grooming apparatus
- (b) grooming of antenna
- (c) grooming of legs
- (d) grooming of abdomen

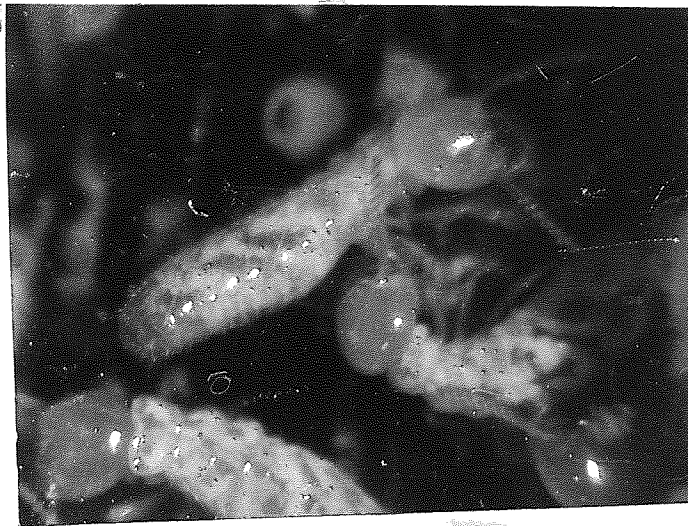


b, c, d next page

b



c



d



- (b) The coating or removal of exudates (pheromones, etc.) from the body surface (Wilson, 1971) which are the basis of the specific characteristic of the termite and the colony. This includes factors like colony odour, which at a gross level is specific to the colony. But within colonies themselves there must exist minor differences between individuals. Grooming reduces such differences by removal of some of the exudates. By ingesting the exudates the termite may achieve a "sharing out" of such individual differences.

Grooming of another individual was carried out by the mouth parts. The lacinia of the maxilla is the chief grooming organ. It is a double toothed heavily sclerotised portion of the maxilla bearing a row of bristles and an area of numerous hairs. Aiding the lacinia in the grooming activities were the other maxillary and labial palps. (Plate 3.3a).

All areas of the termite's body were groomed by nest-mates. When being groomed a termite

- (a) seldom tries to move away, and
- (b) accommodates the groomer by presenting the various regions of its body in an advantageous position. It was often noticed that a passivity was induced in the animal that was groomed (perhaps as a result of the tactile stimulus).

Initiation of the grooming activities was always by the groomer, but termination of the activity was brought about by

either the groomer being (a) violently brushed away or (b) avoided by running away.

Although most commonly two participants were seen in grooming activities, more termites may be involved. One, two, three or more termites may groom a single animal. It is, of course, mechanically impossible for one termite to groom more than one animal simultaneously.

During grooming activities termites may be inclined at different angles to one another at 0° , 90° and 180° . Grooming seemed to occur with equal facility in these various positions, even though the groomer or the groomed may be upside down.

The length of grooming bouts varied from just a few seconds to fifteen or twenty minutes. This seemed to depend on the relative 'mood' of the groomer or groomed to perform the activity.

Grooming can be classified into three categories depending on the region of the body that was groomed.

Region 1: The Head

The areas frequently groomed were the antennae, clypeo-labral region of the mouth, the maxillary and labial palps. The dorsal and ventral sides of the head were occasionally groomed.

- (a) The grooming of the antenna started with the scape or pedicel and progressed along the length of the flagellum. The antenna was held in a groove formed by the labial and maxillary palps while the lacinia scraped each segment of the flagellum. Particles scraped out were normally ingested by the groomer. The antennae were groomed alternately but on occasions the same antenna was groomed twice or thrice before the next one was started, and on each occasion the starting point was the base of the flagellum. (Plate 3.3b).

- (b) Grooming of the maxillary and labial palps followed the same pattern as the antennae, i.e. starting at the base and progressing towards the tip segment by segment.

When cleaning the antennae or the maxillary and labial palps the groomer either stood facing the groomed or alongside it either left or right depending on the side that was groomed.

When grooming the dorsal side of the head or the clypeo-labral region the groomer stood facing the animal and slightly raised its body. The head stooped down on the surface that was to be groomed and the grooming was done by the lacinia in a circular manner sweeping any particles on the surface inwards. The other maxillary and labial palps then swept these particles into the stomodeal opening for ingestion.

When grooming the underside of the head capsule the participation of the groomed termite was needed. The onset of

grooming acted as a cue for the animal that was groomed to stretch and lift its head outwards and upwards. This allowed the groomer to reach all areas of the ventral surface.

Region 2: The Legs

The legs, like the antennae, were subjected to intense grooming. When grooming the legs the groomer stood facing the appendages that were to be groomed and grooming always started at the joints and worked gradually towards the tarsal claws. Grooming began on any one of the five joints, but the sequence following the start was always the same, i.e. working towards the tip of the leg. The leg that was being groomed was held between the mouth parts and the lacinia was used to scrape off adhering particles. Sometimes the groomed termite accommodated the groomer by lifting its leg off the substrate and by tilting its body in order to expose the basal (coxal) parts of its legs.

The leg to be groomed was observed to be picked at random. Often a leg was groomed two or three times and this was at times followed by the grooming of the other legs. However there was no sequence to this - a termite would often terminate grooming after one or it might continue and groom the second and/or the third leg of a side. (Plate 3.3c).

Region 3: The Thorax and the Abdomen

The thorax and the abdomen were subjected to very intensive grooming. As in all grooming activities the lacinia was extensively used. The lacinia moved in a semi-rotary

movement over the surface of the body, scraping and sweeping all particles and exudates inwards into the mouth. The maxillary and labial palps helped in the sweeping of these particles into the mouth.

The animal that was being groomed often facilitated grooming activity by (a) rotating its body to present inaccessible regions, and (b) stretching and swelling the abdominal areas that were being groomed. This was done by arching its abdomen in the opposite side or probably by the flow of body fluids to particular sections of the body. (Plate 33d)

During all these activities (3.4.2) the antenna was often seen to (a) hover over the animal that was being groomed and (b) palpate the region of the body that was being groomed.

3.5. Trophalaxis or Food Sharing Behaviour

Individuals of R. santonensis, like most termites, exchange nutritive substances or trophallax (the term and its definition is discussed in Chapter 4) by both stomodeal and proctodeal means. Exceptions to this rule in the order Isoptera is the Termitidae which only exchange food through the stomodeam.

In R. santonensis the food that was exchanged by either method was of two types.

- (a) a clear fluid, which at times may be a little opaque and viscous, and
- (b) a semi digested, pastelike material.

Principal donors of the stomodeal food in this termite are the 'workers' (from the third stage onwards). On occasion larvae of the second stage have been observed donating stomodeal food. However it was not possible to ascertain whether this material was actually regurgitated material or excess food sticking out of the mouth that was removed by the 'workers'. In this study none of the other castes, i.e. replacement reproductives, the soldiers and 1st stage larvae were observed to donate stomodealy.

All castes were observed to donate proctodeal food. (An analysis of the food exchange among the different castes is described in Chapter 4.).

In previous studies (Grasse, 1949; Grasse and Noirot, 1945; McMahan, 1963 and Alibert, 1963) of food exchange, emphasis had been placed more on rates of transfer rather than on detailed behavioural elements involved in food exchange. The latter is important in understanding some causal factors involved in the two principal acts involved in food sharing. These two acts were:-

- (a) the act of soliciting or accepting food, and
- (b) the act of donating or regurgitating and 'defaecating' food.

The following description was made mainly from observations on workers. Minor variations in the pattern in other castes are recorded elsewhere (Chapter 4.).

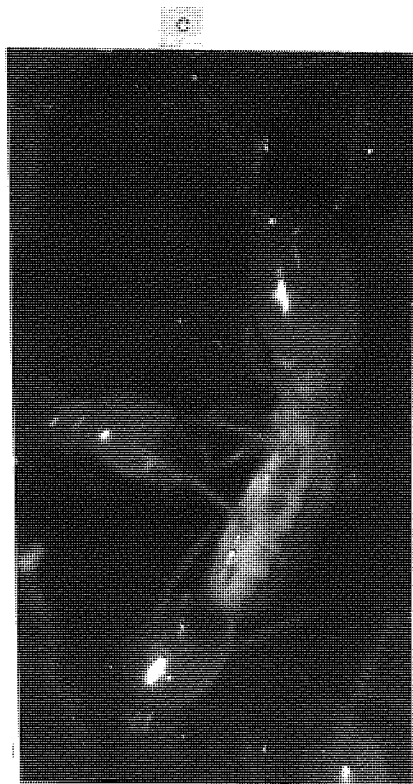
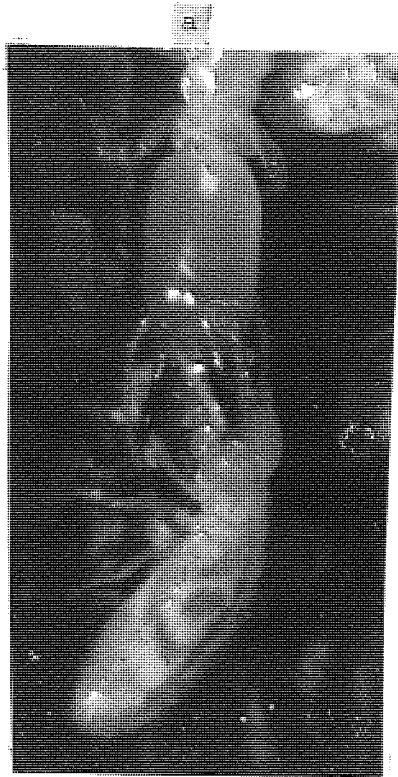
3.5.1. Soliciting or Accepting

Feeding Stomodeally from another Termite

A termite may feed from another termite without exhibiting any palpatory movements of the antennae. The solicitor generally made an orientation movement of the head by raising it out of the horizontal. The body was supported by its legs and the angle to the horizontal varied from small to large so that body axis was almost vertical. Acceptor termites have been observed feeding while on a vertical side wall of the nest and even upside down while clinging to the glass nest roof. The antennae were directed towards and convergent on the mouth parts or head of the donor. The mandibles were always open slightly and food was taken in by the maxillary

Plate 3.4. Food Exchange

- (a) stomodeal exchange: from worker to soldier
- (b) proctodeal exchange: from worker to worker
- (c) proctodeal exchange: from soldier to worker
- (d) proctodeal exchange: from larva to worker



and labial palps which received and directed the food into the stomodeal opening. (Plate 3.4a).

Feeding Stomodeally from another Termite with Antennal Palpation

Feeding from another termite may be preceded and also accompanied by palpation of that termite with the antennae. The antennae were frequently palpated alternatively and they were directed to beat down the front of the head. Sometimes the antennae and/or the ventral side of the donor's head was palpated though the latter only occurred when the orientation of the feeding termite was at right angles to the donor. In order to attract a potential donor to orientate towards and donate, the solicitor on occasion palpated other parts of the body.

Unsuccessful Antennal Palpation

Not all feeding attempts with antennal palpation are successful. The potential donor may fail to respond and when this happens the attempt was referred to as an unsuccessful antennal palpation.

Feeding Proctodeally from Another Termite

Like stomodeal feeding a termite may feed from another proctodeally, without any antennal palpation. The solicitor generally stretched its head towards the perianal region and might 'hold' the tip of the donors abdomen with its mouth parts. The body of the solicitor was supported by its legs on the horizontal and was most frequently, directly behind the donor. However at times the solicitor was at right angles

to the donor on the horizontal or was alongside the potential donor with its mouth parts oriented towards the perianal region. The antennae were directed towards, and converged, on the posterior abdominal region of the donor. The mandibles were slightly open while the palps of the maxilla and labium held or stroked the perianal region (which causes a defaecatory reflex - Grasse et al, 1945) and aided in directing the evacuated food into its own stomodeal opening. (Plate 3.4b, c and d).

Feeding Proctodeally with Antennal Palpation

Similarly to the stomodeal feeding situation, proctodeal feeding from another termite may be preceded and also accompanied by palpation of that termite with the antennae. The antennae were directed to beat down the posterior dorsal and dorso-lateral regions of the abdomen either alternately or simultaneously.

Proctodeal feeding, once initiated by the solicitor was never unsuccessful. If the potential donor's rectal pouch contained no solid matter, a colourless droplet of the liquid contents of the rectal pouch was voided. However an unwilling donor often ran away from a solicitor without donating before initiation began. It was also not uncommon for a donor to turn around immediately on the evacuation of its rectal pouch and 'snatch' back the food donated from the acceptor.

3.5.2. Regurgitating or Donating

Donating Food

A donor termite may donate food to another without showing any palpation movements of the antennae.. When donating the donor raised and stretched its head and held it almost in a straight line to the axis of the body. The mandibles were held open slightly and the antennae were held either wide open away from the acceptor or held almost parallel to each other just above the acceptor's head. The labral mouth parts were flipped outwards and the maxillary and labial palps aided in channeling the regurgitated food towards the acceptor especially when regurgitating semi-solid food. When pure colourless liquid food was donated the labial mouth parts were extruded when a droplet appeared at the tip.

Offering

In 'offering', a termite extruded either a droplet of colourless liquid or a pellet of food, had its labrum projecting outwards, and performed masticatory movements with its mandibles. This was followed by orientating towards a potential acceptor by stretching of its head towards that animal. When such a movement was made without being evoked by a potential acceptor soliciting and immediately feeding, it was called "offering". This may eventually be followed by regurgitation when another termite fed, or may be terminated by the would be donor swallowing the offering when no acceptor responds.

Thus it was characterised by:-

- (a) the donor taking the initiative and presenting food, and always by

- (b) an interval during which the donor "signalled" willingness to donate but when no acceptor responded.

Regurgitation with Antennal Palpation

Regurgitation may be preceded and/or accompanied by palpation of the antennae. The pattern appeared to be similar to those shown when palpation preceded or accompanied feeding.

Unsuccessful Antennal Palpation

Unsuccessful antennal palpation was shown when the palpation was not followed by regurgitation because the potential acceptor failed to respond. Its relation to regurgitation may be established by reference to the behaviour immediately preceding or following it.

Proctodeal Offering

Proctodeal feeding was usually initiated by the recipient. However, exceptionally a response observed in a termite that was satiated was "proctodeal offering". It consisted of approaching another individual, especially a replacement reproductive or soldier, and presenting its anal region to be solicited. This it did by facing the potential feeder and making grooming movements of its fore or mid legs and at the same time arching the tips of its abdomen towards the mouth of the acceptor. A willing acceptor then "gripped"/stroked the perianal region of the donor which caused it to evacuate its rectal pouch.

It was not uncommon for a donor to approach several individuals before succeeding. This behaviour was shown irregularly and by some individuals and not by others.

3.6. Carrying other Termites or being carried by them.

R. santonensis workers occasionally carried other workers, replacement reproductives and soldiers especially immediately before, during or after the latter's moulting. First and second stage larvae were normally carried about. None of the other castes were observed doing this. The carrying termite grasped the other animal by either its legs, thorax or abdomen in its mandibles and walked off. Thus
× carrying a fellow termite involved the same motor patterns as carrying debris, brood, etc. (Plate 3.5).

The termite which was carried often froze into immobility and allowed itself to be carried as a rigid object. The carrier carried the other in front of it and throughout the length of this study no termite that was carried ever was seen to suffer any injury as a result of this behaviour.

Plate 3.5. Carrying a larva.



3.7. Oscillatory Movements

When sufficiently excited or disturbed termites exhibited curious oscillatory movements that generally brought about a greatly increased level of activity throughout the colony. In R. santonensis these movements were observed when:

- (a) the chamber was subjected to vigorous movements like banging or tapping accidentally.
- (b) the chamber was opened
- (c) the colony was exposed to sudden strong light, or
- (d) an alien (either a termite of the same species but different colony or a termite of another species) was introduced into the colony, and
- (e) finally, these movements were also observed when freshly injured or dead termites were introduced in the colony and whenever termites met nestmates in runways or behind them during running or walking.

Various names by various authors have been given to the different types of movements. Table 5.1. after Stuart (1969) gives a comparison of the various names given to these movements.

In R. santonensis movement numbers two and three are most frequently observed. Movement one was sometimes observed among soldiers when disturbed.

Table 5.1. The Terms applied to three well known termite movements.

Move- ment nos.	Authors		
	various: e.g. Goetsch (1936) Emerson (1929) Morgan ((1959)	Stuart(1960,1963)	Howse (1964,1965)
1	Head tapping, head banging	Head tapping movement	Vertical Oscilla- tory movement
2	Nudging, jitter- ing, quivering, jerking	Horizontal recip- rocating movement	Longitudinal Os- cillatory movement
3	Alarm movement, running, dancing to all sides	zig-zag bumping movement (alarm)	Complex oscillat- ory movement.

A number of hypothesis as to the purposes of these movements have been put forward. Notable among them are:

- (a) Emerson (1929) who stated that the movements either released an odour or a normally undetectable vibration.
- (b) Stuart (1963) failed to find any odour associated with the movements but found evidence of a trail laying substance with them. He also found that movements two and three merge or grade into one another. In this he concurred with Goetsch (1936).
- (c) Howse (1965) arrived at the same conclusion as Stuart with regard to the movements being associated with scent trails, but his analysis of cinematographs showed that the Complex Oscillatory Movement (No. 3)

was distinct from the Longitudinal Oscillatory Movement (No. 2). The former was normally associated with large disturbances (alarm) and the latter with short-lived low intensity stimuli.

These movements were obviously associated with alarm and though they played a significant part in the behavioural repertoire of R. santonensis, no detailed investigations were undertaken in the present study.

All the castes except the first stage larvae were observed performing these movements.

involved in this behaviour.

3.8. Behaviour during Courtship and Copulation

Courtship, if defined to include all behaviour from pairing to excavation of the initial cell where copulation is accomplished, is restricted to winged imagoes only. Buchli (1960) recorded a detailed analysis of this in R. lucifugus (Rossi). In replacement reproductives certain components of this chain of events were seen to occur, in the present study. They were observed on two occasions. On both occasions the female was seen to initiate the behaviour by approaching the male and taking a 'calling' attitude (abdominal tip slightly raised). The male responded by moving backwards towards the female, until after a few movements sideways the tips of their abdomens came together, and insemination occurred accompanied by two or three jerks on the part of the male. Though actual contact was maintained for less than thirty seconds the whole episode took up to two minutes.

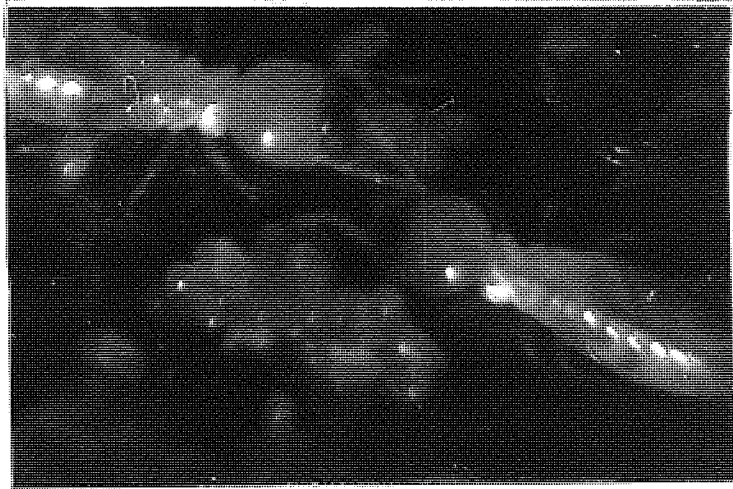
None of the other castes was involved in this behaviour.

3.9. Care of Eggs and Newly Hatched Larvae.

All activities associated with egg and larval care were carried out by workers only. This consisted mainly of:

- (a) Transporting of eggs from one place in the chamber to another (perhaps to shield eggs from vibration, light, heat, excess humidity).
- (b) Cleansing of the surface of the eggs periodically.
- (c) Coating of egg surfaces with 'salivary secretions'.
- (d) Piling of eggs in a cluster, i.e. by collection of each one from wherever it had been laid, transporting it, coating it with saliva and sticking it to the rest of the cluster. (Plate 3.6).
- (e) Helping the new larvae during egg eclosion.
- (f) Feeding of young larvae - i.e. by regurgitating of clear colourless salivary liquid to the brood in the first few days of larval life; this pattern was similar to the regurgitation of food to older larvae and other castes.
- (g) Grooming brood - similar to grooming older individuals.
- (h) Brood carrying and brood retrieving.

Plate 3.6. Care of eggs.



3.10. The Disposal of Injured and Dead Nestmates

All activities associated with the disposal of injured, dying and dead nestmates were carried out by 'workers'. This consisted mainly of :

Cannibalism:- When a badly injured nestmate was chewed little by little, while still alive, till it was completely eaten. (Plate 3.7a).

Necrophagy:- When a dead nestmate was eaten, and

'Walling up':- When a cadaver was, buried with wood fragments, oral and anal cement, when found to be unpalatable. (Plate 3.7b).

A detailed account of the above behavioural patterns is further given in Chapter 6.

Plate 3.7. Disposal of injured and dead.

- (a) an injured (still live) worker being cannibalised.
- (b) an unpallatable cadaver being walled up
(blotched around cadaver are wood fragments
and faecal material).

a



b



FORM 4-233C

CHAPTER IV

1. The first part of the chapter is devoted to a discussion of the various types of data which are available for the study of the history of the United States.

2. The second part of the chapter is devoted to a discussion of the various types of data which are available for the study of the history of the United States.

3. The third part of the chapter is devoted to a discussion of the various types of data which are available for the study of the history of the United States.

4. The fourth part of the chapter is devoted to a discussion of the various types of data which are available for the study of the history of the United States.

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8. The eighth part of the chapter is devoted to a discussion of the various types of data which are available for the study of the history of the United States.

9. The ninth part of the chapter is devoted to a discussion of the various types of data which are available for the study of the history of the United States.

10. The tenth part of the chapter is devoted to a discussion of the various types of data which are available for the study of the history of the United States.

11. The eleventh part of the chapter is devoted to a discussion of the various types of data which are available for the study of the history of the United States.

12. The twelfth part of the chapter is devoted to a discussion of the various types of data which are available for the study of the history of the United States.

13. The thirteenth part of the chapter is devoted to a discussion of the various types of data which are available for the study of the history of the United States.

14. The fourteenth part of the chapter is devoted to a discussion of the various types of data which are available for the study of the history of the United States.

15. The fifteenth part of the chapter is devoted to a discussion of the various types of data which are available for the study of the history of the United States.

CHAPTER 1V

TROPHALLACTIC BEHAVIOUR4.1. Introduction

Trophallaxis or the mutual exchange of liquid food, plays a very important role in the social organization of insect societies (Wheeler, 1928). Wilson (1965, 1971) has summarized the historical background and definition of the term. He points out that the term has become a 'panchreston' and instead of meaning the single phenomenon of liquid food exchange has also come to mean recognition of colony mates (Emerson, 1929); inter individual communication (Ribbands, 1952, 1953 and 1965); distribution of pheromones involved in caste differentiation (Pickens, 1932; Light, 1942-1943; Miller, 1942; Lüscher, 1955, 1958, 1960, 1961, 1962 and 1964; Karlson and Butenandt, 1959; Butler, 1963); caste elimination (Lüscher, 1958) and the transfer of gut-living symbiont protozoa (Grasse and Noirot, 1945). It is perhaps unnecessary to discuss the exact definition of the term 'trophallaxis' here except to state that definitions are best when used as specific terms and in this study trophallaxis is used as it was defined originally, i.e. "exchange of nourishment" (Wheeler, 1918 as quoted by Wilson, 1971).

The process of trophallaxis among termites has been studied by Grasse and Noirot (1945); Noirot (1952); Gosswald and Kloft (1958); Gosswald (1962) on Kaloterme flavicollis (Fab), McMahan (1963-1966) on Cryptoterme brevis, Walker and Alibert (1960-1965) on K. flavicollis and

Cubitermes fungifaber (Sjöstedt), among others. Harris and Sands (1965) and McMahan (1969) have discussed the role and implication of trophallaxis in termite societies. Almost all of the above mentioned studies looked at food transfer patterns of the different castes and tried to elucidate the role of trophallaxis in the production and maintenance of the various caste ratios in a termite society. But an integral part of food exchange is the various movements (already described in Chapter 3) shown by the soliciting and donating individuals. These movements have seldom been studied. The work described in this chapter is an attempt at analysing these movements, especially in the 'worker' termites.

4.2. Food transmission in *R. santonensis*.

As there was little information on the feeding patterns and feeding capacities within *R. santonensis* societies it was decided to look into this first.

4.2.1. Patterns of Food Exchange in *R. santonensis*

Members of all cellulose feeding lower termites that depend on gut symbionts for the digestion of their food, practise both stomodeal and proctodeal exchange of food. The transmission of this food in most of the termites studied was centred around the 'workers' who acted as principal donors, although the reproductives of incipient colonies have been known to provide food to the young (McMahan, 1969). Noirot et al (1969), in their review of the nutritive regimes of termites stated, that the functional and replacement reproductives of all termites received stomodeal food and Alibert (1960) found that reproductives of *K. flavicollis* donated proctodeal food to young workers and in turn received proctodeal food from the young as well.

Soldiers generally received (from workers) solid and liquid stomodeal food (Noirot, 1969) and though no mention was made of their donating abilities by Noirot, McMahan's (1963) and Alibert's (1969) studies on the Kalotermitidae showed that soldiers have a low but a real capacity to donate proctodeal food.

Among the larvae, and nymphs (workers) after the second instar, acceptance and donation both stomodeally and

proctodeally occur regularly. Larvae of the 3rd and 4th instar were recognized by Alibert (1969) as the best recipients and donors of trophallactic food. As already mentioned the younger larvae (1st and 2nd instar) of most species received both stomodeal and proctodeal food and McMahan (1969) stated that these young larvae of K. flavicollis were capable of donating proctodeal food, at least in incipient colonies.

4.2.2. Observations on R. santonensis

To elucidate the feeding patterns in a R. santonensis society, direct observations were made on randomly selected individuals of the different castes, in plate cultures.

McMahan (1963-1966) and Alibert (1959-1965) have been using radio-isotopes to understand feeding associations and especially their influence on the integration of termite societies. While the isotope-tracer technique lends itself well to unravelling the distribution of pheromones, etc., it cannot always yield conclusive results when used to study trophallactic behaviour of individuals. Some of the problems as pointed out by McMahan (1969) are:

- (a) Nuclides often are excreted (lost) through the cuticle-- and this can distort data of stomodeal and proctodeal exchange of food;
- (b) Nuclides can be acquired, besides trophallaxis, through ingestion of defaecated material, through the body wall (When in contact with food impregnated with radioactive isotopes), through grooming; and

- (c) "social insects are generally sensitive to effects of radiation and little is known about the change in their behaviour as a result of even low level irradiation.

Besides these, highly expensive radiation detecting equipment is needed, if any meaningful results are to be had from using radio-isotopes.

Termite individuals selected for observations were marked and were observed for ten hours (almost continuously except for two 30 minute breaks). All food exchanges were recorded for analysis as described in Chapter 2.4).

Results

Patterns of Food Exchange

None of the replacement reproductives, soldiers and young larvae (1st and 2nd instars) were ever observed to feed directly on the wood-flour available in the plates, though reproductives were observed frequently to imbibe water from the substrate. The dependence of the above individuals on the 'workers' of the colony, has of course, been well documented, in other lower termites as well.

All members of the colony participated in food exchanges. Fig. 4.1 illustrates the pattern of food flow in the plate colonies. There was a free flow of food among individuals of the different castes. However this free flow of nourishment followed certain paths which can be classified into (a) The stomodeal flow and (b) The proctodeal flow.

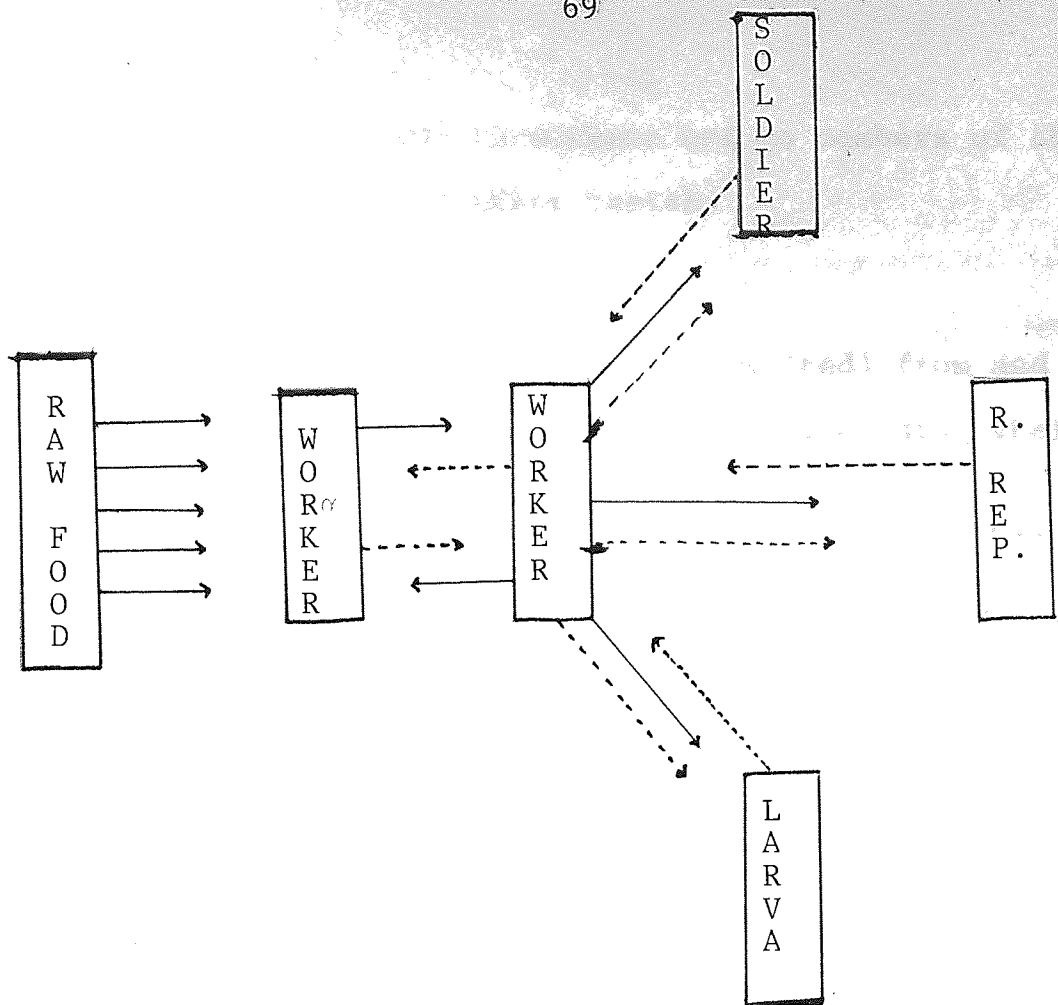


Fig. 4.1. Patterns of Food Transmission in a *R. santonensis* colony.

—————→ Stomodeal
 -----→ Proctodeal

(a) The Stomodeal Flow:

(i) The 'workers' formed the focal point of all stomodeal exchange, i.e. they accepted from and donated stomodeally among themselves and donates to all other castes.

(ii) The other castes, i.e. replacement reproductives, soldiers and larvae accepted (solicited) food only from the workers, and they donated (proctodealy and not

stomodealy) only to workers not to members of their own castes or to other castes.

(b) The Proctodeal Flow:-

- (i) The 'workers' accepted (solicited) from and donated proctodealy to all castes including their own.
- (ii) The secondary reproductives accepted (solicited) from and donated proctodealy to 'workers' only.
- (iii) The soldiers accepted (solicited) from and donated proctodealy to 'workers' only.
- (iv) The larvae (1st and 2nd stage) accepted (solicited) from and donated (only 2nd stage) proctodealy to 'workers' only.

The Frequency of Trophallaxis of the Different Castes

The data recorded consisted of the number of exchanges (acceptance or donation either stomodealy or proctodealy) made by the individual under observation. No record was made of the different types of food (semi-solids or liquids) exchanged. However in the case of workers, all feeding on raw wood flour from the substrate was ignored (since direct feeding is not "food exchanged").

There was a great variation in the frequency of exchanges made by individuals of the different castes. Table 4.1 gives the results of the number of times a given individual either accepted (solicited) or donated stomodealy or proctodealy from or to another individual of the colony.

Routes of exchange

A 't' test was done on the data in table 4.1 to establish if there was any difference in the routes (stomodeal or proctodeal) of exchange taken by the different castes.

Workers:

There was no significant difference in the manner in which 'worker' termites accepted their food from colony mates ($t = 1.26$, and table $t = 2.021$ at $p = 0.05$) and there was also no difference in the manner in which workers donated to their colony mates ($t = 0.79$, and table $t = 2.021$ at $p = 0.05$).

Larvae:

As far as acceptance behaviour was concerned larvae showed no significant difference between the stomodeum or proctodeum ($t = 0.73$ and table $t = 2.145$ at $p = 0.05$) and as for donation none of the larvae observed ever donated stomodeally.

Soldiers:

The soldiers under observation showed a highly significant preference for accepting food stomodeally ($p > 0.01$).

Like larvae, soldiers did not donate stomodeally.

Replacement Reproductives:

All the replacement reproductives that were observed showed a highly significant preference for stomodeal food ($p > 0.01$).

All donations done by the reproductives were proctodeal.

Further analysis of the data was carried out to establish:

Table 4.1. Frequency of trophallaxis in a ten hour period (0800-1800) by individuals of different castes/instars in a R. santonensis colony.

	WORKER				LARVAE (Stg.1 & Stg.2)				SOLDIER				REPLACEMENT REPRODUCTIVE			
	ACCEPTANCE		DONATION		ACCEPTANCE		DONATION		ACCEPTANCE		DONATION		ACCEPTANCE		DONATION	
	Stom.	Proc.	Stom.	Proc.	Stom.	Proc.	Stom.	Proc.	Stom.	Proc.	Stom.	Proc.	Stom.	Proc.	Stom.	Proc.
	6	5	14	7	4	5	0	6	5	0	0	1	10	2	0	14
	2	12	4	3	11	13	0	7	6	3	0	3	14	3	0	12
	15	2	5	5	9	2	0	5	6	2	0	2	14	2	0	11
	10	6	7	5	4	7	0	9	3	3	0	4	16	5	0	15
	8	4	7	0	7	10	0	4	5	2	0	3	13	3	0	12
	9	8	9	1	8	15	0	6	4	3	0	2	11	4	0	11
	7	18	13	16	7	4	0	9	6	2	0	3	15	1	0	11
	7	6	1	13	6	6	0	7	6	3	0	3	15	5	0	11
	8	10	14	3	4	8	0	5	4	3	0	3	12	5	0	13
	9	8	4	6	8	4	0	6	6	0						
	3	7	5	6	6	6	0	8	8							
	11	8	9	8	12	1	0	8	4							
	13	11	5	4			0	0	0							
	6	6	8	6			0	0	0							
	7	10	14	10			0	0	0							
	7	4	4	6			0	0	0							
	13	4	14	5			0	0	0							
	8	8	7	10			0	0	0							
	9	4	12	9			0	0	0							
	8	8	2	2			0	0	0							
Total	121	157	158	125	86	74	0	80	39	18	0	21	105	25	0	99
n	20	20	20	20	12	12	12	12	8	8	8	8	8	8	8	8
\bar{x}	8.3	7.45	7.9	6.25	7.166	6.166	-	6.66	4.87	2.25	-	2.625	13.125	3.125	-	12.375
S.error	+0.7	+0.81	+0.96	+0.88	+0.75	+1.13	-	+0.47	+0.39	+0.37	-	+0.32	+0.72	+0.52	-	+0.53

- (a) The difference (if any) in the frequencies of the two functions, i.e. donation and acceptance within each caste, and
- (b) the difference (if any) in the frequencies of the two functions, i.e. donation and acceptance between the castes.

Table 4.2 gives the frequencies of food exchange both donation and acceptance by individuals of the different castes in a plate colony. A 't' test was done to compare the mean frequencies of acceptance and donation in:

(a) Workers:

Though individual workers showed great variation between acceptance and donation during the period of observation, the population as a whole showed no significant difference (at $p, 0.05$) between acceptance and donation.

(b) Larvae:

Almost all the larvae observed showed more instances of acceptances than donations - the difference was highly significant ($p > 0.001$).

(c) Soldiers:

Like the larvae all the soldiers observed showed more instances of acceptance than donation. The difference in the mean was highly significant ($p > 0.001$).

(d) Replacement Reproductives:

Only one of the eight reproductives accepted less and donated more during the period of observation. But a comp-

Table 4.2. Frequency of trophallaxis (Acceptance vs. donation in a ten hour (0800-1800 period by individuals of different castes/instars in a R. santonensis colony.

	WORKER		LARVAE (1 & 2)		SOLDIER		REPLACEMENT REPRODUCTIVE	
	Acceptance	Donation	Acceptance	Donation	Acceptance	Donation	Acceptance	Donation
	11	21	9	6	5	1	12	14
	14	7	14	7	9	3	17	12
	17	10	11	5	8	2	16	11
	16	12	11	9	6	4	21	15
	12	7	17	4	7	3	16	12
	17	10	23	6	7	2	15	11
	25	29	11	9	8	3	16	11
	13	14	12	7	7	3	17	13
	18	17	12	5				
	17	10	12	6				
	10	11	15	8				
	19	17	13	8				
	24	9						
	12	14						
	17	24						
	11	10						
	17	19						
	16	17						
	13	21						
	16	4						
Total	278	283	160	80	57	21	130	99
n	20	20	12	12	8	8	8	8
\bar{x}	15.7	14.4	12.5	6.67	7.13	2.63	16.25	12.38
s.devi.	3.99	5.84	2.11	1.61	1.25	0.92	2.51	1.51
s.error	± 0.9	± 1.31	± 0.61	± 0.47	± 0.44	± 0.32	± 0.89	± 0.53

arison of the means indicated that the frequencies of acceptances were more than donations. The difference was significant ($p > 0.01$ but < 0.001).

Finally a comparison of the capacities between the different castes was made to establish the differences in their rate of trophallaxis. Since the data to be analysed were from inherently independent samples (castes) the Mann-Whitney U* test was employed to test the significance of the differences in trophallaxis between the different castes.

Acceptance

Workers:

The 'workers' accepted (solicited) significantly more than the larvae ($U = 56$, $p.0.05$) and soldiers ($U = 0$, $p.0.05$); showed no significant difference in the acceptance behaviour when compared to the replacement reproductives.

Larvae:

As stated above larvae accepted significantly less than 'workers'; less than replacement reproductives ($U = 21$, $p.0.05$) and significantly more than soldiers ($U = 0.05$, $p.0.05$).

Soldiers:

Soldiers accepted significantly less than workers, larvae (as stated above) and reproductives ($U = 0$, $p.0.05$).

* Ref: Siegel, S.: Non Parametric Statistics for the Behavioural Sciences (p. 116-126).

Replacement Reproductives:

There was no significant difference in the frequencies of acceptance by reproductives when compared to the workers, but when compared to larvae and soldiers they accepted significantly more:

Donation

Workers:

There was no significant difference between workers and reproductives when it came to donor activity. But workers donated significantly more than soldiers ($U = 0$, $p.0.05$) and larvae $U = 12$, $p.0.05$).

Larvae:

As stated above the larvae donated less than workers. They also donated significantly less than replacement reproductives ($U = 4$, $p, 0.05$), but donated significantly more frequently than soldiers ($U = 4.5$, $p. 0.05$).

Soldiers:

The soldiers donated significantly less than workers (see above), larvae (see above) and reproductives ($U = 0$, $p.0.05$).

Replacement Reproductives:

There was no significant difference in the frequencies of donations between workers and reproductives. But reproductives donated significantly more than larvae and soldiers.

4.2.3 Conclusion

It can be concluded from the above preliminary study that:

- (1) Food exchange in R. santonensis was an open system where the 'workers' collected and consumed the raw material from natural sources and exchanged this material with an unlimited number of nestmates of all castes.
- (2) No other castes (except reproductives and replacement reproductives of incipient colonies) were capable of consuming food from natural sources and were hence dependant on the workers for their nourishment.
- (3) Like all other lower termites food exchange was carried through stomodeal and proctodeal methods.
- (4) Though the other castes (young larvae, soldiers and replacement reproductives) did not consume raw food, they were still actively involved in donating food and this food was donated to workers only.
- (5) All castes were capable of soliciting food from stomodeal and proctodeal openings of the donors; however, except for the 'workers' and immature larvae, the other castes showed a preference for soliciting from the stomodeum. This is probably due to the fact that (a) like most lower termites, the bulk of the proctodeal material is made up of protozoa with little protein in R. santonensis, (Noirot and Noirot-Thimothee, 1969) and (b) since the castes other than 'workers' and immature larvae are almost at the end of their development (moulting) regimes, there is little need for them to replenish their gut symbionts lost during ecdysis, by soliciting proctodeal material. Therefore, it seems probable that the difference in the nutritional value between the two foods is responsible for the evolutionary

development of preference for stomodeal food, in the dependant castes. It is clear from the present study that

(6) Donations made by all castes other than workers was also clear that trophallaxis was not done primarily through the proctodeum.

(7) It is worthwhile to note that reproductives generally received food more often than immature larvae and the larvae more often than soldiers. Likewise reproductives were solicited more often than larvae and larvae more often than soldiers. Further investigations would have to be carried out, to establish whether this differential food exchange forms the basis of caste ratios or is merely a result of the varying gut volumes of the different castes.

4.3. Stimulus Situation Releasing Food Sharing Behaviour

It was quite clear from the preceding section that in R. santonensis the 'workers' played a principal role in trophallaxis. It was also clear that trophallaxis was not a simple phenomenon, but a complex behavioural pattern involving the two distinct acts of acceptance (soliciting) and donation. The work described in the following section attempts to analyse this phenomenon in the 'workers' of R. santonensis.

Although R. santonensis (like most lower termites) which donate both proctodeally and stomodeally can be physically capable of accepting and donating simultaneously, this does not commonly occur. Different factors must influence the individual termite into either accepting (soliciting) or donating; thus: hunger or food deprivation might increase acceptance (soliciting) behaviour, and 'satiation' increase donor behaviour.

'Workers' of termite families that take solid food from the substrate tend to feed well beyond their needs. The cibarium and fore gut of these 'workers' are designed so that they can contain a large accumulation of food. It is through the large dilatory cibarium that food is passed little at a time into the foregut. It is also the cibarium that acts as a reservoir for regurgitated food for feeding the other members of the colony. The mechanism has been described by Vishnoi (1956). Like the cibarium, the hind-gut is also even more capable of dilation (Noirot et al, 1969) and thus capable of containing large volumes of semi-

digested food to donate to colony mates. Thus it seems that such a digestive system has clearly been evolved so that food can be distributed by fed individuals to unfed individuals - thus bringing about the integration of the colony by sharing food.

4.3.1. Initiation and Termination

McMahan (1963) stated that in Cryptotermes brevis, where food exchange was mostly proctodeal, the exchange was normally initiated by the acceptor (solicitor), although prospective donors were infrequently observed to seek out potential acceptors to donate to. She also stated that termination of the bout was usually brought about by the recipient, but under exceptional circumstances donors have been known to terminate a feeding bout. Grasse (1949) described the donor's part as a passive one where it reacted only to the stimulation of the acceptor.

It had already been established that R. santonensis workers exchanged food with equal facility stomodeally and proctodeally. An analysis of all the food exchanges performed by workers (in a normal colony) was carried out to find out if R. santonensis workers showed any particular pattern of behaviour in relation to initiation and termination. The data are given in Table 4.3 below.

Table 4.3. The relationship between initiation and termination in acceptors (solicitors) and donors of R. santonensis workers.

	Acceptor		Donor		Total
	No.	%	No.	%	
Initiate	180	57%	135	43%	315
Terminate	136	47%	152	53%	288

The results showed that, in the food exchanges observed, acceptors tended to initiate the bout more often than donors and donors tended to terminate the bout more often than acceptors ($\chi^2 = 3.84$; $p < 0.05$ at 1df). However as the table showed, up to 43% of donors initiated food exchanges and up to 47% of acceptors terminated it. It seems reasonable to suggest that the factor that determines whether a donor or acceptor shall initiate trophallaxis may depend on the relative strength of motivation* in the potential participants. Thus if a potential donor is motivated relatively more strongly than the potential acceptor, then the former is more likely to make the first movements initiating a bout of food sharing.

In experiments, described later, in which the tendency of workers to accept food had been (experimentally) increased by starvation, it was noticed that in a great number of cases (>75%), trophallaxis was initiated by the hungry acceptors. In such cases the potential acceptor goes actively soliciting from potential donor to donor - raising its head towards

* N.B. Ref. pg. 114 for definition of motivation

their mouth or anus and/or palpating them with its antenna either on the head (if soliciting stomodealy) or the perianal region (if soliciting proctodealy). Such behaviour often evokes the regurgitatory/defecatory response from the potential donors.

In colonies with plenty of food it was frequently noticed that potential donors showing high tendencies to donate were moving from nestmate to nestmate offering food, by projecting the food bolus out of the mouth and presenting the mouth part to the potential acceptor (when food exchange was done stomodealy) or by presenting the anal region (when food exchange was done proctodealy). This then induced the potential acceptors to feed from the donor. In a satiated colony it was not uncommon to observe a potential donor approaching another termite and offering food and in turn having food offered to it.

As shown above, trophallaxis was more often terminated by the donor, termination by the acceptor was not greatly less common. This again seemed to depend on the relative strength of motivation of the participants. In a stomodeal exchange the donor or acceptor terminated by withdrawing its head and turning away; in a proctodeal exchange the acceptor terminated by withdrawing its head or the donor terminated by breaking the exchange with a jerk

4.3.2 Length of Bouts

The range of bout lengths varied from 1.5 seconds to 15 seconds. Table 4.4 gives the mean range of bout lengths

Table 4.4. Mean duration of food exchange between individuals of a R. santonenensis colony

		B O U T L E N G T H					
From	To	STOMODEAL		PROCTODEAL			
		n	Mean duration in seconds	S. Error	n	Mean duration in seconds	S. Error
worker	worker	214	4.83	± 0.09	198	4.00	± 0.15
worker	larvae	99	7.01	± 0.50	28	9.80	± 1.0
worker	soldier	37	4.33	± 0.31	2	5.85	± 1.05
worker	replacement reproductive	78	4.33	± 0.28	16	5.46	± 0.72
larvae	worker	-	-	-	44	3.44	± 0.22
soldier	worker	-	-	-	25	3.89	± 0.35
replacement reproductive	worker	-	-	-	59	3.42	± 0.17

seen in a normal R. santonensis colony. Thus, it seemed that this termite species, at least, limited its food exchange to short durations. Bout length did not appear to indicate the relative hunger or satiation of the participants. The more hungry a particular individual was the greater the number of exchanges, not their duration.

4.3.3. Number of Participants

Trophallaxis in R. santonensis most frequently involved two participants. However on a number of occasions one donor was observed to donate to two individuals (one stomodeally and one proctodeally). It is, of course, impossible for one termite to receive food from two donors at the same time since acceptance is stomodeal only.

4.3.4. The Stimulus Situation "Releasing" Food Sharing Behaviour.-----

Free (1956) analysed the various 'releasers' of trophallactic behaviour among honeybees and came to the conclusion that the following factors were important in stimulating trophallaxis:

- (1) Colony 'odour' of the participants.
- (2) The 'smell' of the head and antenna.
- (3) Movement, in releasing donating behaviour - it did not play any part in releasing acceptance behaviour.

But Montagner (1966, as reviewed by Wilson, 1971) who repeated some of Free's experiments on the social wasp Vespula (Paravespula) germanica and V.(P) vulgaris found

that though the head attracted other workers who were prepared to engage in food exchange the inert head was not sufficient to release begging or offering behaviour. There was some regurgitation when artificial wire antennae fixed to the severed heads were vibrated at 20 to 100 cycles per second - but this did not last for more than several seconds. Trophallaxis was sustained only when the participants engaged in continuous reciprocal signalling.

From continuous observation of worker termites it was clear that food sharing in R. santonensis occurred in a number of stimulus situations and there was no specific posture or movement that was essential to release either the begging or donating response. It was found that correct, i.e. "own" colony odour was an important factor in bringing about trophallaxis.

Movement of the participants was never found to be necessary to release either acceptor or donor behaviour, although antennal touch from a prospective participant increased the chances of trophallaxis. Likewise the exact topographical position of the head seemed to be unimportant, as food sharing was seen to occur with equal facility in various positions, i.e. the participants could be inclined at different angles to one another. Not infrequently they were seen engaged in food exchange when 'upside-down' to each other.

Thus it was seen that food sharing in R. santonensis occurred when:

- (1) A termite seeks out another and presents food.
- (2) A termite meets another 'en route' and offers it food.
- (3) A termite seeks out another and solicits food.
- (4) A potential acceptor antenally palpates a potential donor, which then presents food.
- (5) A potential donor antenally palpates a potential acceptor and presents it with food.

Various experiments reported below were done to investigate the problem further.

4.3.4. Experiments with models:

Free's (1956) and other experiments were repeated using various models. The results are tabulated below in Table 4.5.

Table 4.5. Worker response to models.

Type of model	Response
1. Severed head stuck on a pin (fresh)	cannibalised
2. Severed head plus thorax (fresh)	cannibalised
3. Severed posterior region of abdomen (fresh)	cannibalised
4. Head and thorax of a corpse	necrophagéd
5. Abdomen (posterior) of a corpse	necrophagéd
6. Head plus thorax (with movement)	Few (4) attempts by others to donate but ultimately cannibalised.

In all the experiments the models were ultimately consumed by colony mates and hence did not provide any meaningful information. Cannibalism was elicited in all cases where there was fresh haemolymph (see Chapter 6).

and the results are tabulated below

4.3.4. Amputation of the antennae.

The loss of one of its antenna, does not seem to affect the normal trophallactic behaviour of R. santonensis workers. The following experiment was conducted to look at the function of the antenna in food exchange. Termite workers were randomly selected from plate cultures for the experiment, and their antennae were amputated by surgical scalpels while they were immobilized by chilling. As fresh haemolymph provoked colony mates into cannibalizing the injured organ - the experimentals were isolated from the rest of the colony (in chamber C of the plate nests) for 24 hours. During this isolation the experimentals were also deprived of any food and water. Food deprivation was found to increase the frequency of acceptance and depress donation. During the period of isolation of the experimental the rest of the colony was confined to Chamber A of the nests (where there was plenty of food). The middle chamber was free of termites and was also empty of food. At the end of the 24 hour period the experimental and the rest of the colony were allowed to mix in Chamber B. This prevented the experimental from obtaining its food and water from the substrate and hence it was obliged to solicit from colony mates.

The number of food sharing acts carried out by the experimental, on reintroduction with the rest of the colony

was recorded on tape over a period of 2 hours observation, and later analysed. Ten worker termites were subjected to this experiment. Ten controls were also set up, they were subjected to the same starvation/isolation period, but their antennae were not amputated. The results are tabulated below in Table 4.6.

Table 4.6. Effect of antennal amputation on food exchange*.

	EXPERIMENTALS No. of Attempts*			CONTROLS No. of Attempts*		
	Unsuc- cessful	Succe- ssful	Total	Unsuc- essful	Succe- ssful	Total
1	9	1	10	13	17	13
2	12	4	16	7	10	17
3	12	4	16	10	15	25
4	11	10	21	9	17	26
5	13	1	14	6	8	14
6	10	4	14	8	8	16
7	6	1	7	6	12	18
8	6	0	6	7	10	17
9	13	3	16	10	17	27
10	11	10	21	9	13	22
Total	103	38	141	85	127	212
n	10	10	10	10	10	10
mean	10.3	3.8	14.1	8.5	12.7	21.2
s.dev.	2.5	3.5	5.1	2.1	3.6	5.5
s.error	± 0.7	± 1.0	± 1.6	± 0.6	± 1.1	± 1.7

* Includes donation as well as acceptance - in almost all the cases only acceptance took place during the period of observation.

The table shows:

- (i) Antennal amputation reduced the total amount of food sharing behaviour. The average number of attempts made to feed were: experimentals 14.1, controls 21.2.
- (ii) The antenaless termites were also less successful in their attempts to feed than controls. When a χ^2 test was done on the totals for successful and unsuccessful attempts, it was found that the difference between the two groups was highly significant ($\chi^2 = 36.94$, $p > 0.001$ for 1 d.f.), i.e. the controls were more successful in their feeding attempts than the experimentals.

Thus it was seen that the antennae played an important role in food exchange. However, some food sharing was achieved even without them. Failure to bring about food exchange in the absence of antennae seemed to be due in most cases, to failure to recognize* potential donors, and when food was offered, failure to orientate correctly to the donors, such as positioning the mouthparts at the right position.

The maxillae and mandibles were not subjected to any special study. They probably play no part in 'releasing' trophallactic behaviour, though they might help in channeling the food into the mouth.

*N.B. Recognition of nestmates in termites as well as in most social insects is brought about by distinct colony odours. This is further discussed in Chapt. 5.

4.3.5. The Internal Factors:

Although the antenna, odour, etc., were of importance in bringing about food exchange, clearly it was the internal factors that played a critical role in determining whether a given individual showed acceptor or donor behaviour. These factors are not clearly understood yet, but can include the peripheral sensory receptors of the digestive system. Acceptor behaviour was probably affected by the factors which produced hunger and donor behaviour by the degree of satiation. Unfortunately there is little information on the sensory physiology of termites. This has been reviewed by Richard, (1969) but the review does not contain any information on the sensory physiology of 'feeding'. Free, (1957) stated that bees about to give food generally had more food in their honey stomachs than those about to receive it and Vowles (1955) suggested that donor behaviour in ants was very much controlled by the degree of crop distension.

In order to study the importance of the internal conditions on acceptor and donor behaviour twelve worker termites were starved of food and water in Chamber C of the plate culture for twenty-four hours. After the starvation period the experimentals were allowed to mix with 'fed' individuals of the same colony in Chamber B of the plates (this prevented the starved individuals from feeding directly on the natural cellulose). They were then observed continuously for six hours and records were made of all the food exchanges carried out by them. Only records of successful attempts were made and the combined results of the

twelve are given in Table 4.7. Four individuals selected randomly from the nest were also observed continuously for six hours and the food exchanges carried out by them were also recorded. These were treated as controls.

The changes in food sharing exhibited by the experimentals are graphically illustrated in Fig. 4.2. The experimentals as a whole showed only acceptor behaviour in the first hour, though two did, in fact, donate once each during the first hour. This was an exception as most of the experimentals avoided potential solicitors. Maximum acceptance was shown in the first hour and this changed very little during the next two hours. But during the fourth hour, the frequency of acceptance dropped almost to half of the first hour. From then on it fluctuated between two and three times in the hour.

Donor behaviour was not seen in the first hour except the two cases mentioned above. Experimentals that were solicited avoided donating and solicited in turn. But donor behaviour was shown from the second hour and increased thereafter to the fourth hour by which time the experimentals donated as much or more than they accepted.

The controls under observation showed no significant difference between acceptance and donation from hour to hour during the six hours of observation.

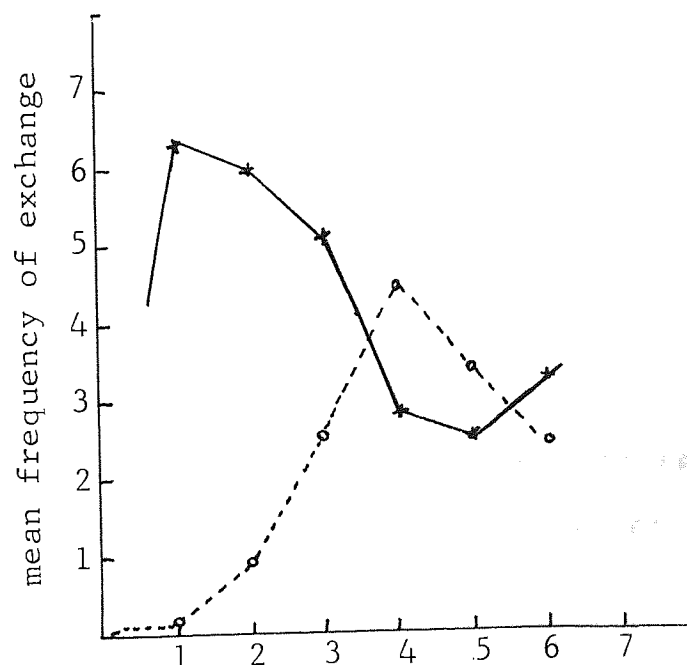
Table 4.7. The changes in food exchange between starved and unstarved termites

	No. of Experimentals	Hr. in chamber after starvation	ACCEPTANCE						DONATION						Between Acceptance & donation
			Stomodeal	Proctodeal	Total Ex-change	Mean No. Ex-change	Std. Dev.	Std. Error	Stomodeal	Proctodeal	Total Ex-change	Mean No. Ex-change	Std. Dev.	Std. Error	
STARVED INDIVIDUALS	12	First	52	24	76	6.33	2.42	+0.69	2	0	2	0.17	0.39	+0.11	8.8*
	12	Second	50	22	72	6.0	1.91	+0.55	8	3	11	0.92	0.71	+0.23	6.19*
	12	Third	50	12	62	5.17	2.41	+0.69	11	20	31	2.5	1.17	+0.34	3.56*
	12	Fourth	24	10	34	2.83	0.94	+0.27	39	27	66	4.5	2.32	+0.67	2.35*
	12	Fifth	23	7	30	2.5	1.51	+0.44	18	24	42	3.42	1.38	+0.39	1.61 ⁺
	12	Sixth	28	11	39	3.25	0.75	+0.22	12	17	29	2.41	0.90	+0.25	2.67*
CONTROLS UNSTARVED	4	First	6	7	13	3.25	1.70	+0.85	4	6	10	2.5	1.29	+0.65	0.70 ⁺
	4	Second	6	4	10	2.5	2.64	+1.32	8	6	14	3.5	2.38	+1.19	0.81 ⁺
	4	Third	9	8	17	4.25	1.71	+0.85	6	7	13	3.25	2.63	+1.31	0.64 ⁺
	4	Fourth	4	7	11	2.75	1.25	+0.62	2	3	5	1.25	1.25	+0.62	1.70 ⁺
	4	Fifth	6	8	14	3.5	1.29	+0.64	7	4	11	2.75	2.5	+1.25	0.53 ⁺
	4	Sixth	5	6	11	2.75	1.25	+0.62	4	8	12	3.0	1.83	+0.91	0.22 ⁺

* Significant at P 0.05

+ Not significant at P 0.05

Fig. 4.2. Changes in the Feeding Behaviour (i.e. either donation or acceptance) of starved individuals on re-entry to colony



successive one hour periods

o-----o Donation
 x-----x Acceptance

4.3.6. Conclusions

From the preceding study a few conclusions can be drawn, viz. that the important stimuli evoking food sharing can be classified into two groups:

- (i) The stimuli that facilitated either donor or acceptor behaviour in particular, e.g. the internal stimuli from the digestive system.
- (ii) The stimuli that facilitated food sharing in general but are not specific to either donor or acceptor behaviour and these included the following:

The antennae played the most important role in orientating to the correct position for food exchange and in 'recognizing' the other termite by odour, for unless the other termite was 'recognized' food sharing was not shown. Movement was not found to be essential nor was topography of the body.

The above were simply facilitatory factors. It was not possible to locate any specific stimuli that acted as a "specific key" unlocking a "specific lock". There were (antennal) palpatory movements but they were not specific in evoking either acceptor or donor behaviour - the palpation seemingly acted in an excitatory way, lowering thresholds and not as releasing mechanisms such as were reported in bees (Free, 1956) and wasps (Montagner, 1966).

4.4. The Significance of Antennal Palpation

Antennal palpation was shown by donors and solicitors alike. Observation of fully fed individuals indicated that these palpatory movements were strong during the early parts of their donatory behaviour but grew weaker gradually with successive donation. In the case of hungry individuals the antennal palpation preceding or accompanying soliciting behaviour was strong initially but as the termite gradually got satiated the movements tended to become weaker and eventually disappeared altogether. Individuals showing no palpations at all tended to alternate between donor and acceptor behaviour.

Wilson (1971) stated that foreleg stroking in ants during food exchange was like the antennal play of honey bees and indicated strong motivation. It occurred more frequently in the acceptor.

This led to the question as to whether such mechanisms operated in Reticulitermes as well, i.e. whether antennal palpatory movements accompanying or preceding food exchange were an expression of the relative strength of motivation? Various experiments were done to answer this question and these are reported below.

4.4.1.

(a) Tendency to solicit food induced experimentally

Starving of termites increases their soliciting behaviour - but preliminary studies showed that when starving termites were subjected to a period of dessication as

well, their soliciting behaviour showed a dramatic increase. However over-desiccation inevitably caused death and hence, for experimental purposes, termites were subjected only to mild desiccation, and only individuals that appeared to be "healthy" (termites with outstretched antennae and moving about actively were considered "healthy", while those with curled up antennae, shrunken bodies and moribund were considered "unhealthy") after desiccation were observed for trophallactic behaviour.

Individual workers which showed active food exchange under normal conditions were selected and these were left in open petri dishes which were then placed in a desiccator containing saturated aqueous solutions of $K_2CO_3 - 2H_2O$. This gave an approximate R.H. of 43% at $24^{\circ}C$ (ASTM Recommended Practice for maintaining const. R.H.). The dessicator was deliberately not tightly sealed so as to make conditions less severe, in order to obtain relatively active experiments.

Apparently "healthy" individuals were selected and these were allowed to move into Chamber B of the plate nests into which the rest of the colony (well fed) was also moved (see Chapter 2 for technique), from Chamber C. The entrance to Chamber C was then shut for the rest of the observation period, thus forcing the experimental to obtain all its food from colony mates and not from natural sources.

(b) Soliciting Behaviour of the Experimental

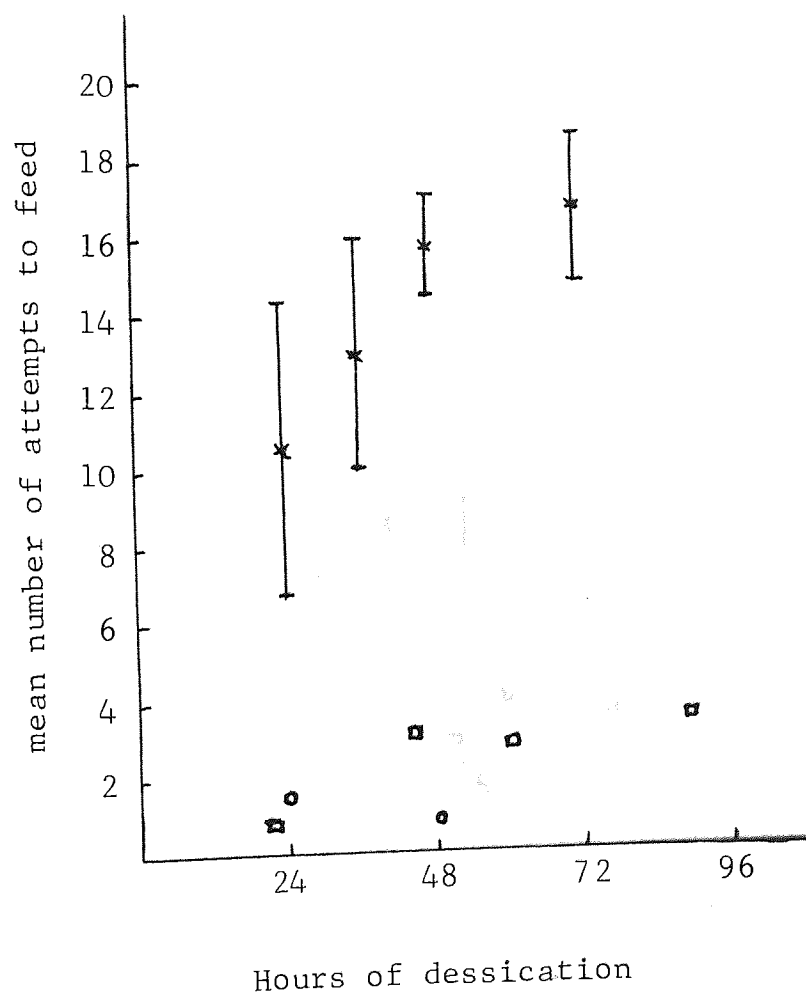
The food sharing behaviour of the experimentals (starved and desiccated for varying lengths of time) returned to the nest was observed for 60 minutes from the time of their return. The data were recorded on tape for analysis later. The well fed nest mates provided a reservoir of food from which the experimentals could draw. A control group of termites isolated for 24 hours but not starved or desiccated was used to check the results of isolation alone.

Results: The frequency of food exchange carried out by the experimental termites is shown graphically in Fig. 4.3a and Fig. 4.3b.

Fig 4.3a shows that there was an increase in the soliciting behaviour with increasing period of desiccation. However this rate of increase slowed down after 60 hours of desiccation and as mortalities due to desiccation increased rapidly after 72 hours observation was not carried out on experimentals desiccated beyond 60 hours.

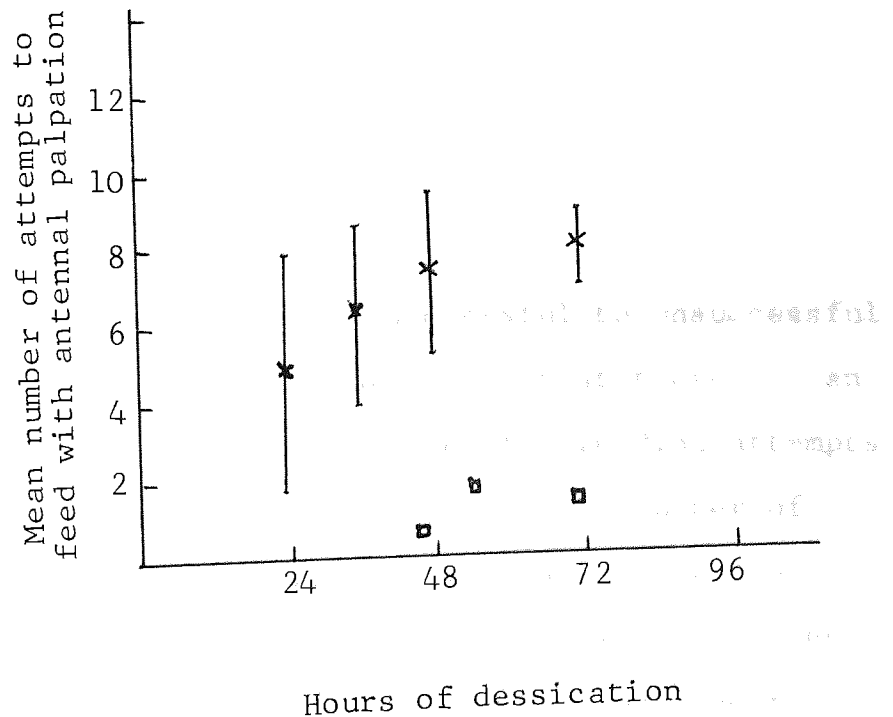
The next figure (4.3b) clearly shows that there was a steady increase in the amount of feeding with antennal palpation with increasing periods of desiccation and this increase was closely parallel to the total number of feeding attempts. Both the figures (4.3a and 4.3b) thus showed that a large proportion of the total attempts to exchange food was made up of soliciting (as opposed to donating) and soliciting with antennal palpation (as opposed to

Fig. 4.3a. The effect of varying periods of dessication on the frequency of attempts to feed.



Controls ○ Donation
 □ Acceptance

Fig. 4.3b. The effect of varying periods of dessication on the frequency of attempts to feed with antennal palpation.



Controls o

soliciting without antennal palpation).

Some donor behaviour was shown by the experimentals much later during the observation. This was confined to the 24 - 36 hour group.

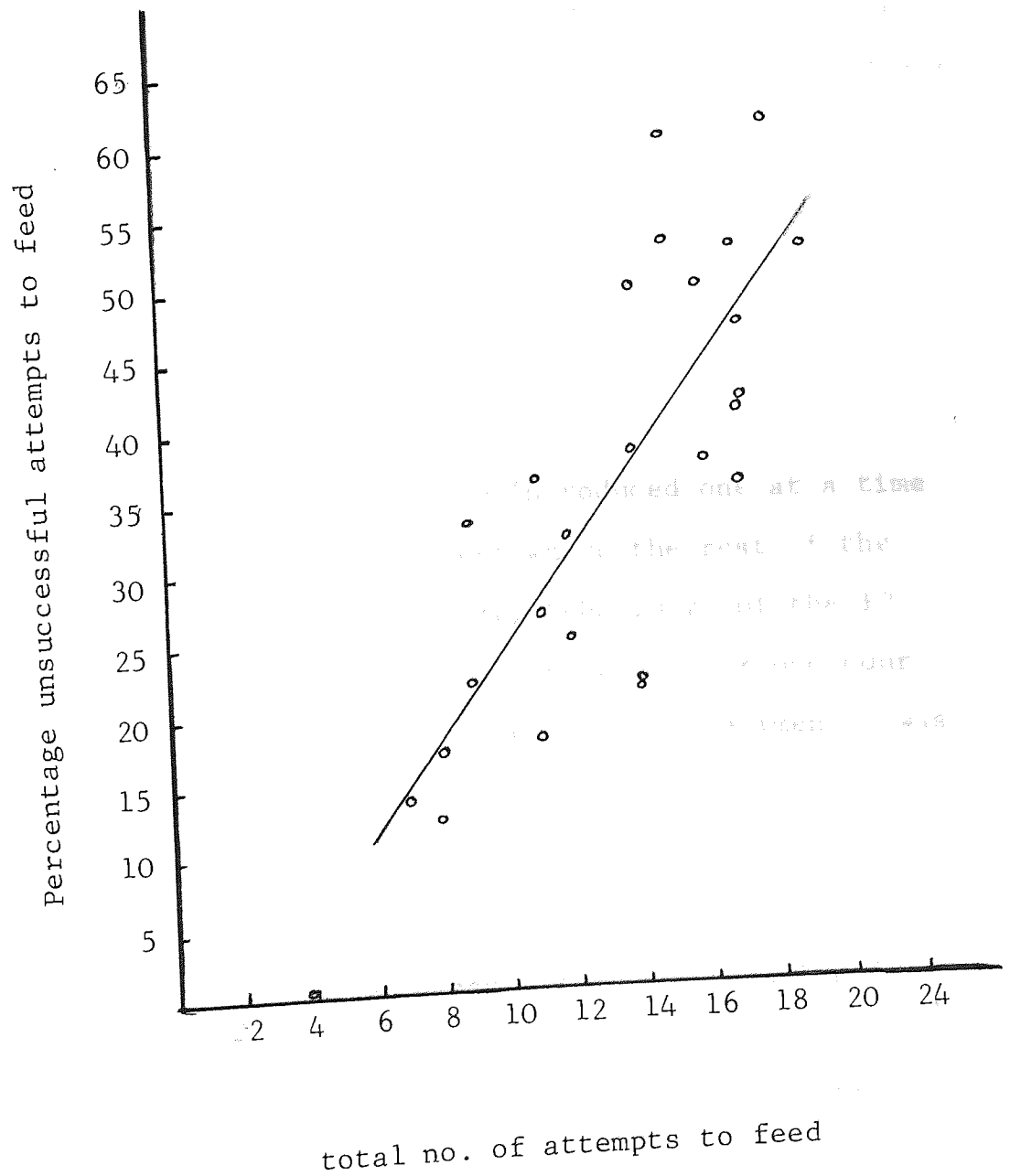
There were some attempts to exchange food without any palpation. These were once again found in the 22 - 36 hour groups which showed a high peak for such behaviour.

The control groups showed some soliciting and donating behaviour (see Fig. 4.3a and 4.3b).

Fig. 4.4 shows the proportion of unsuccessful feeding to the total number of attempts to share food and by implication compares the proportion of successful to unsuccessful food sharing attempts. The figure shows that there was an increase in the percentage of unsuccessful feeding attempts with increasing desiccation. Further, as the number of feeding attempts increased with the increasing duration of desiccation so too did the percentage which were unsuccessful. The whole of this increase appeared to be linear ($r = 0.816$). This probably was the result of workers which were much more active in food sharing spending less time in waiting for a potential donor to respond before passing on to another one and soliciting from that.

Thus it can be concluded that soliciting with antennal palpation and the total number of successful feeding attempts increased together with increasing duration of desiccation

Fig. 4.4. The relationship between total attempts to feed and the percentage of unsuccessful attempts.



(until it reached a peak at 72 hours when high rates of mortalities prevented the collection of meaningful results).

Soliciting behaviour without antennal palpation did not seem to increase with increased duration of desiccation. It in fact showed a peak at the 24 - 36 hour period, and hence at fairly low levels of the tendency to feed. Thus the experiment suggested that soliciting with palpation occurred when the tendency to feed was high and soliciting without palpation occurred when the tendency to feed was lower. To test this further the experiment described below was carried out.

Experiment:

Twelve termite workers were subjected to desiccation from 36 - 60 hours. These were introduced one at a time into Chamber B of the plate nest where the rest of the colony was enclosed. The feeding behaviour of the 12 individuals was then continuously observed for one hour from the time of introduction. Only one experimental was used for this study in any one day.

Results:

Table 4.8 gives the frequency of food sharing (all acceptance) carried out by the experimentals in four successive 15 minute periods during the one hour observation.

Table 4.8. Change in the frequency of food exchange with time

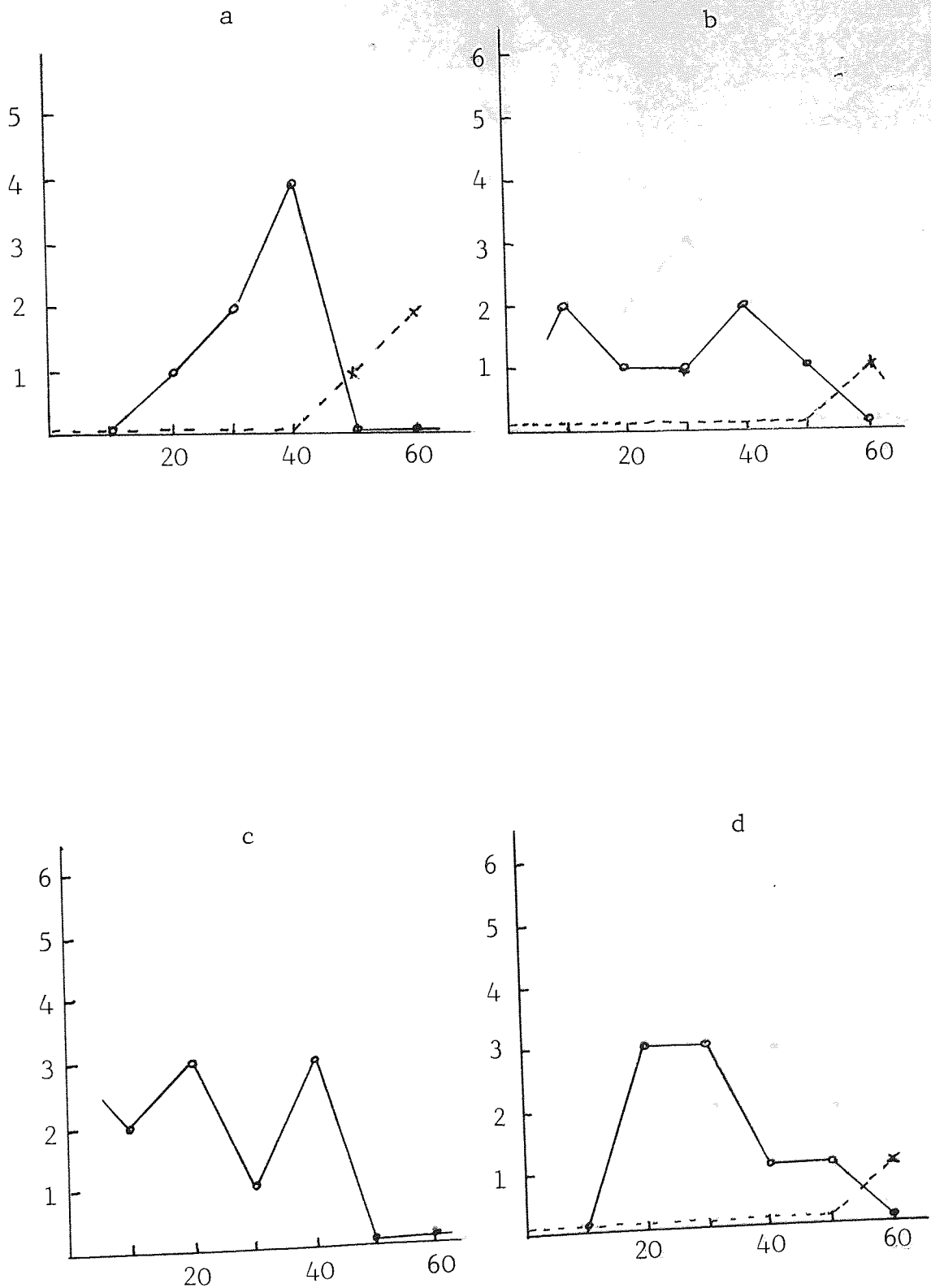
	1st 15 Mins.	2nd. 15 Mins.	3rd. 15 Mins.	4th. 15 Mins.
Total Exchange	31	31	23	12
Mean No. of Exchange	2.58	2.58	1.92	1.0
Std. Dev.	1.38	0.90	1.16	0.95

Though the tendency to exchange food declined over the hour, there was no significant decline in the number of exchanges between the 1st and 2nd 15 minute periods. One possible explanation for this can be that on the initial return of the experimental from isolation much time was spent in the examination and grooming of the returned worker (thus depressing the frequency of food exchange). But overall there certainly was a decline over the hour in the soliciting behaviour of the experimentals.

Fig. 4.5a to 4.5l shows the changes in the method of soliciting for food by the 12 individuals over the hour. The results were considered individually because lumping the 12 together tended to obscure the information, due to individual variation. The feeding movements were considered in successive 10 minute periods.

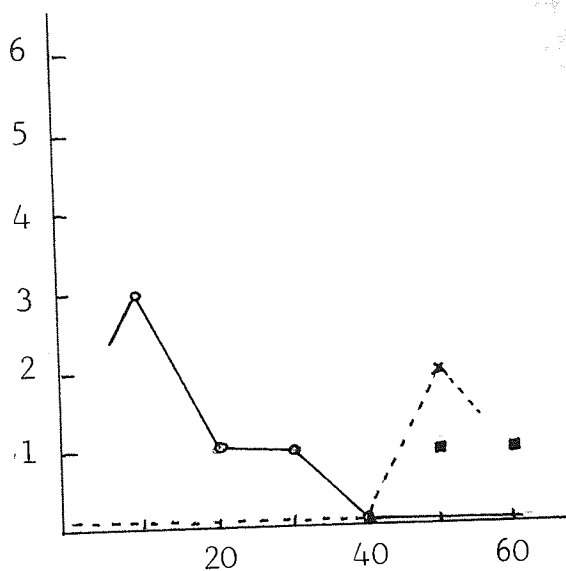
The graphs showed that feeding with antennal palpation was shown in all of the 12 records and feeding without palpation was shown in 8 of the 12 individuals. In all of those 8 individuals feeding without palpation was shown only towards the end of the observation period, the peak

4.5. Changes in food sharing behaviour

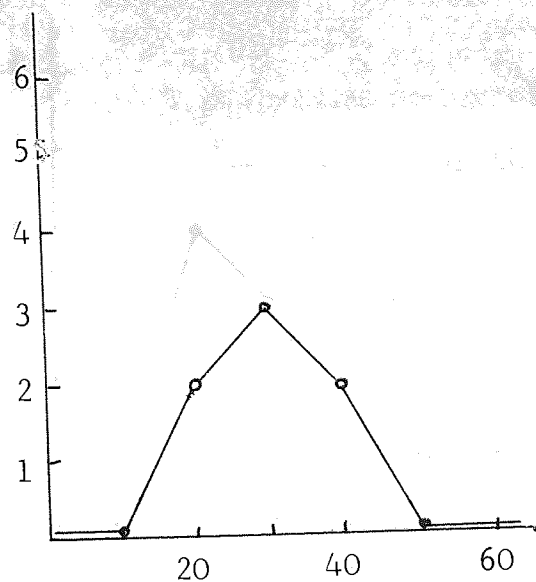


x-----x food sharing (soliciting) without palpation
 o-----o food sharing (soliciting) with palpation
 • Donation
 abscisse = successive 10 minute period
 ordinate = frequency of food sharing.

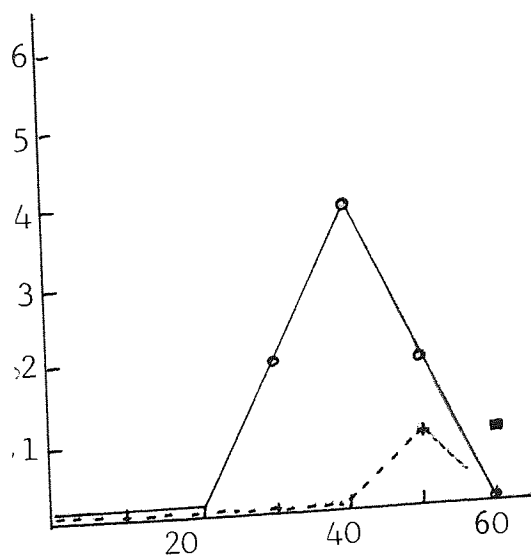
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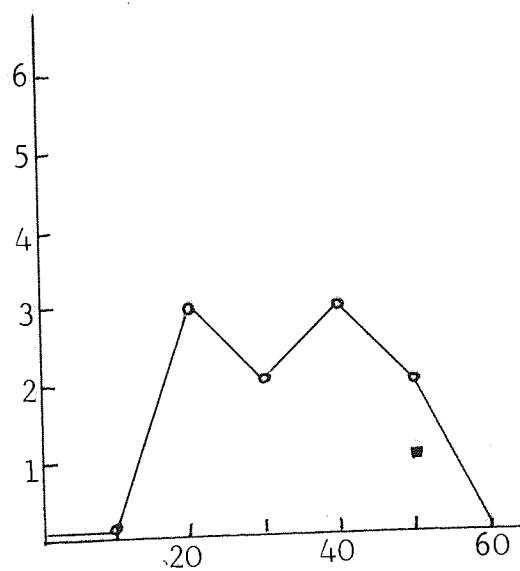
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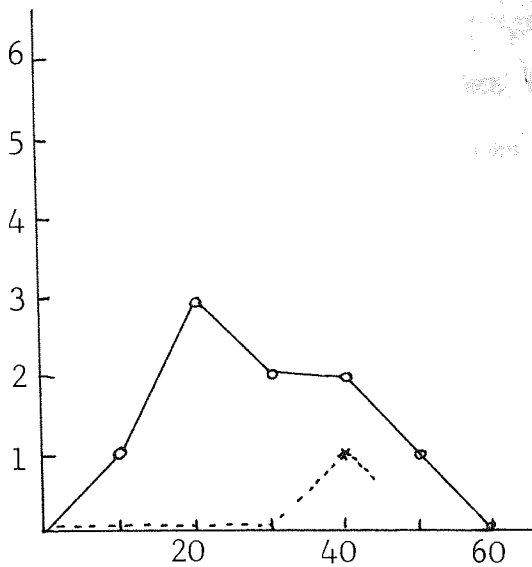
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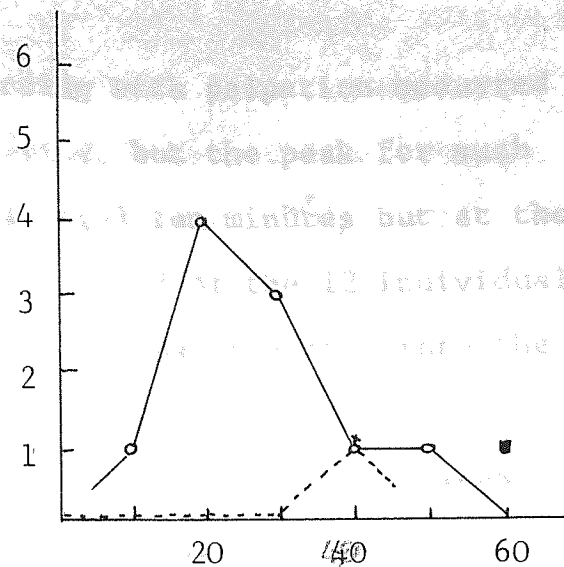
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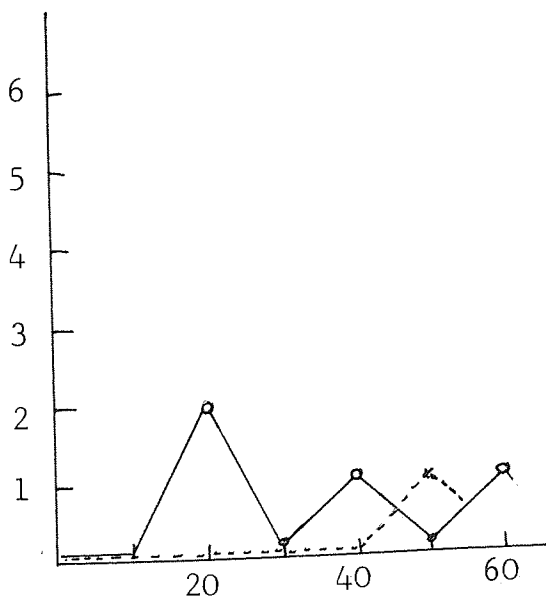
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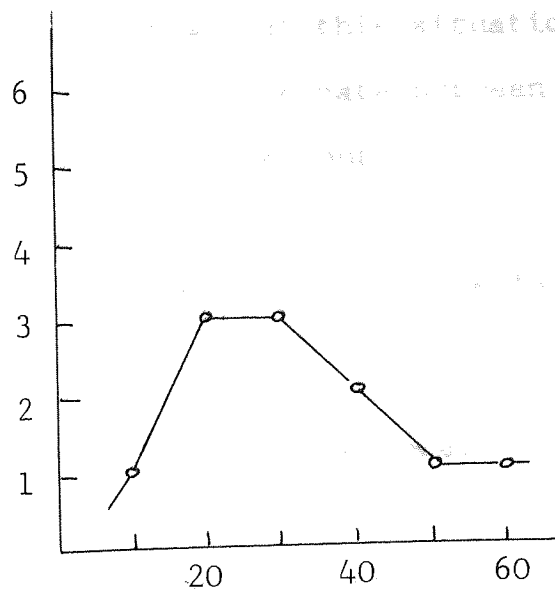
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was observed to occur between the 50 -60 minute period.

It can be seen that feeding with palpation occurred throughout the observation period, but the peak for such feeding was seen not at the initial ten minutes but at the 20 - 30 and 30 - 40 minute periods (8 of the 12 individuals). This may have been due to the fact that re-entry into the nest initially depressed food sharing (grooming, etc.) but when once soliciting (feeding) behaviour was released from the inhibition, it tended to build up rapidly to a peak (20 - 40 minute period) and slowed down gradually towards the end of the hour when feeding without palpation started building up.

It was at this latter period that donor behaviour was observed in the experimentals (in 4 of the individuals) when tendency to feed had fallen. It was at this situation that the termite workers may be seen to alternate between soliciting and donating even after a single bout.

Thus it can be concluded from the above experiments that:-

- (1) The bulk of feeding behaviour was with antennal palpation
- (2) when tendency to feed was high it was accompanied or preceded by antennal palpation and when it was low feeding occurred without any palpation.
- (3) when tendency to solicit (feed) was low, donor behaviour was seen.

4.5. Discussion

The flow of food in R. santonensis colonies followed the same path as other lower termites, i.e. the workers collected material from natural sources and distributed this among themselves and also to the other castes and immature young. At the same time the immature young and the other castes, though incapable of extracting food materials from natural sources, contributed to the total trophic picture of the colony by proctodeal donation to the workers, which once again distributed this food material to the rest of the colony.

It was, however, difficult to compare the rate of food exchange in a R. santonensis colony with those of C. brevis or K. flavicollis. (McMahan (1963), who studied the former, and Alibert (1959), who studied the latter, both used tracer techniques. Radioactive material carried by one or more workers in a colony travels in a branching chain pattern within the colony and is a useful tool to unravel the rapidity at which food material or other substances are transmitted within a colony from a source. It, however, did not take into account the feed back of the same material to the donors in return and hence did not specifically show the quantity of food material an individual termite accepted from and donated to its nestmates. Prolonged direct observation, as has been used in this study, does enable the investigator to estimate by inference (frequency of exchange x time taken per exchange) the amount of food received by an individual and the amount donated by it. Three major drawbacks of this method of investigation were:

- (1) It was not possible to assess accurately the exact quantity of food exchanged.
- (2) It was not possible to identify the type of material exchanged, i.e. undigested, semi-digested or 'synthesized' (secretions of salivary glands, anal glands, etc.) food.
- (3) The experimental colony must be able to exist 'normally' in a plate culture, for continuous observation.

Frequency of food exchange in termite colonies can be influenced by a number of factors, viz:

- (1) Colony age, size, caste-ratio and the presence of the royal pair (Alibert, 1968).
- (2) Relative humidity, temperature and time (Gosswald and Kloft, 1963).
- (3) Pre-moulting periods, when food exchange is depressed or totally absent (McMahan, 1966).

Colony size in this study was limited to 200 individuals made up of workers, reproductives and soldiers (see Chapter 2). When observations were carried out the colonies were already reproducing and there were a number of young larvae present as well. The experimentals were selected at random and they were observed at the same time every day.

The pattern of distribution of food material in the colonies was not wholly equitable - 'workers' were more

active in food exchange than the other castes, they accepted food more often and donated more often as well, than the other castes. This result seemed to be in line with the observations of McMahan and Alibert on C. brevis and C. flavicollis respectively. Replacement reproductives were given more food than any of the other castes by the 'workers'. Alibert (1968) found that in C. flavicollis the diet of the reproductives varied with their age and the size of the colony. Young reproductives fed mainly on salivary food, and when amidst small groups (1, 2, 10) they fed on a little proctodeal food as well, and finally when amidst 30 or more individuals they fed on proctodeal food regularly. Replacement reproductives in this study received more stomodeal food than proctodeal food and since the study was restricted to a very short period it was not possible to study the possible changes in diet with age and colony size. However, it is worthwhile observing that Allen (1955, 1956) and Taranov and Ivanov (1946) reported that the rate of egg production of the honey bee queen was dependant on the amount and type of food she received from the nurse bees. No doubt it was equally necessary for reproductives in termites, which also lay great numbers of eggs, to receive large quantities of nutritive material. The very young (1st and 2nd stage) larvae received food more frequently than the soldiers in R. santonensis and the soldiers least of all. Soldiers were often inactive in an undisturbed colony and thus would have a much slower metabolic rate than a growing larvae or a reproducing queen. Whether the quantity or quality of food given an individual caste or instar was directly

correlated to their needs has not been investigated at all, but this should throw light into the distribution of food among the different individuals in a colony.

In as far as donation was concerned the workers, being the only caste capable of feeding directly on raw cellulose, donated more frequently than any of the other castes. However the frequency of their donation was not significantly greater than that of the replacement reproductives. Alibert (1968) observed that in Calotermes the royal couple contributed greatly to the trophic picture of the colony by donating frequently, thus confirming the results obtained in this study. Soldiers donated least of all, even less than the young (1st and 2nd stage) larvae.

The difference in the donating capacity of the different castes has seldom been explained in literature and based on the present study the following explanations can only be postulated.

(1) That in terms of the quantity of food received the replacement reproductive received the most, the soldiers least and the immature larvae in between. Thus when it comes to donation the replacement reproductives had more to donate than the soldiers. This must be especially so when one considers the fact that 'social rumination' is a way of digestion of food in termites (Harris and Sands, 1965) i.e. food goes through several individuals before it gets finally ejected as faeces. This could also explain why the immature larvae with a faster metabolic rate and shorter gut donate more frequently than soldiers.

(2) The inhibition theory of caste differentiation seems to require that all castes serve as donors of pheromone-bearing food material (Lüscher, 1962; Miller, 1969). This is consistent with the fact that in a population of R. santonensis there is always a greater number of soldiers than reproductives and hence for the transmission of sufficient amounts of their respective inhibitory substances the individual reproductive would have to donate more frequently than the individual soldier.

As described in Chapter 2, food exchanges in termites, as in the other social insects, involved the two factors of donating and soliciting. In R. santonensis at least the internal factors seemed to play a key role in determining whether a worker termite acted as a donor or an acceptor. When the worker was fully fed it acted as a donor and when it was deprived of food it solicited food. A worker could be shifted from a predominantly acceptor behaviour to a predominantly donor behaviour by merely feeding it. Similar behaviour was reported for Formica by Wilson (1971) and Free (1959) observed that in honey bees the role which a given worker assumed depended very much on the state of its crop content, i.e. bees about to give food generally have more food in their honey stomachs than those about to receive it. In R. santonensis donating workers were generally individuals that had accumulated a considerable amount of food in their gut. It would be interesting, but was not possible in the present investigation, to assess the quantity of food in the gut of a donor and in that of a recipient.

Besides internal factors the odour of the participants seemed to play an important role in bringing about food exchange. Food donation to isolated individuals, on re-entry to the colony, was often suppressed during the initial period but gradually picked up with the isolate 'adsorbing' the colony odour. Unlike bees, where head and antenna (Free, 1956), and wasps, where interplay of antennae between the participants (Montagner, 1966), were found to act as releasers for food exchange, R. santonensis workers were found to use their antennae to identify potential donors and acceptors and not as releasers of food exchange. However the lack of antennae, although it depresses frequency, does not prevent the termite from participating in food exchange. Termites use their antennae in facilitating food exchange and as shown by Grasse and Noirot (1945), antennal palpation was used by potential acceptors to stimulate regurgitatory and defaecatory reflexes. In R. santonensis workers antennal palpation was not only used by acceptors but also by donors, though on the whole acceptors were found to use it more often than donors and it was especially seen to occur in termites showing a strong tendency to feed. It is worthwhile to note that Wallis (1961) found that ants expressed strong motivation for food by vigorous foreleg stroking and Wilson (1971) reported that honey bees indicated their motivation by vigorous antennal stroking.

However, one cannot infer motivation on the part of R. santonensis from the study of pure motor patterns such as were observed in the present study. This is because

the same motor patterns were shown as a result of different types of motivation* (e.g. grooming, aggression, etc.). Ethologists studying the behaviour of the higher vertebrates (birds and mammals) have extensively inferred motivation from motor patterns. Responses in the vertebrates may be more amenable to this approach where motivated behaviour has been measured by the intensity or rate of consummatory behaviour, or by the rate or intensity of work the animal will do to reach its goal. Moreover, information is also available on the neurophysiology of motivation (where the parts of the nervous system involved, and the effects produced on them by external stimuli are known) in higher vertebrates (Deither and Stellar, 1961; Hinde, 1966) than on insects in general and termites in particular.

* Motivation is defined as any goal-directed action instigated by a central motivational state, which itself is created by an interaction within the brain between the neural consequences of bodily organismic states and neural consequences of environmental incentives (Bindra, D. and Stewart, J. (1971) in Motivation.

CHAPTER V

1. Introduction

2.

3. Conclusion

CHAPTER VAGGRESSIVE BEHAVIOUR5.1. Introduction

An abundance of evidence has been documented on the inter and intraspecific hostility shown by social Hymenoptera. Though such evidence is few and far in the case of the Isoptera, which also form complex societies like the Hymenoptera, it is not unreasonable to expect them to defend those commodities and communities on which they depend for their survival against competitors. There is some evidence of aggressiveness of termites, especially the soldiers, of a number of species (Fuller, 1915; Grasse, 1939; Noirot, 1955; Bouillon, 1969), against alien species. Andrews (1911) and Nel (1968) found evidence of aggression on the part of termites of the different castes against intraspecific aliens. Howse (1970) stated that termites that wandered into wrong nests were always killed.

While it is easy to postulate a credible system of recognition of other species on genetic variations of any of a number of characteristics such as size, shape, odour and behaviour, it was not easy to suggest a basis for distinction between individuals of different communities of the same species. It has been suggested by a number of workers that this basis of distinction can be odour (Emerson, 1929; Stuart, 1969 and Howse, 1970).

5.2. Behaviour shown to Termites entering a Colony

A termite entering a nest, whether its own or an alien, provokes a series of responses. These responses vary from species to species, between colonies of the same species and between individuals of the same colony. While members of alien species were always killed (Howse, 1970; Stuart, 1969) response to nestmates and aliens of the same species varied from mild examination as in the case of K. flavicollis (Grassi and Sandias, 1893-1894) to outright attack as in the case of Eutermes rippertii Rambler (Andrews, 1911). In the present investigation reaction to intruders varied between the two extremes, depending on the colonies and also on the position of the intruder on being discovered, and the discoverer of the intruder. Aliens often attracted violent attention from soldiers at nest entrances but mild reaction within nests and they evoked mild response from workers outside nests and violent ones within it.

Observations and experiments described below were carried out with the aim of elucidating the response shown by R. santonensis to intruders.

Methods

The experiments were carried out using the plate nests described earlier. The colonies were confined to chamber A of the nests and the experimentals (aliens) were introduced into Chamber C and allowed to find their way to Chamber A. Chamber B and C were devoid of food and substrate and though the residents* were allowed to wander into them, they seldom

*"Residents" refers to termites belonging to the nest under observation.

did this. Normally one or two soldiers of the colony were always found near the entrance of Chamber A. Stuart (1969) remarked that the normal position of soldiers of Zootermopsis, was either around the reproductives or the periphery of the logs they infest.

Observations were made in the following situations:

- (a) an alien termite entering a colony
- (b) a resident termite isolated for 24 hours entering its own colony.
- (c) a resident termite isolated for two weeks entering its own colony, and
- (d) a termite selected at random and observed within its own colony.

Observations were carried out for thirty minutes from the time the alien or experimental was encountered by the first individual. Though longer observations would have been fruitful this would have necessitated the marking of termites which in turn could have been a source of attention. Recordings were made for analysis as described in Chapter 2.4.

Aggressive behaviour in R. santonensis as in all termites is basically a defensive act and may consist of just 'examination' of the alien, or the alien may cause an 'alarm' reaction followed immediately by 'flight', or the alien may evoke a 'threat' reaction from the residents or a 'snapping' 'lunging' behaviour whereby the intruder was injured or immobilized. Injury often resulted in the cannibalism of the intruder.

(1) Examination

Examination of an intruder, especially when it was outside the nest proper was, often carried out by orientation towards the intruder, accompanied by stretching and orientation of the antennæ towards it. But within the nest this was accompanied by touching the alien with the tips of the antennae.

(2) Alarm/Flight

Alarm reaction on discovery of the alien was swift. 'Worker' termites, as described in Chapter 3, carry out a series of vigorous jerking or bumping movements while standing in the same position. This vigorous jerking or oscillatory movement was often followed by flight. Flight consisted of turning away from the alien and running swiftly, often undirected, towards the centre of the colony. Some workers often responded by depositing faecal 'material' between the object causing alarm and the rest of the colony. As Stuart (1969), pointed out, faecal deposition was a reaction to a low intensity alarm.

(3) Threat

The threat posture was very marked in the case of the soldiers. The soldiers raised their heads to the horizontal and both head and antennae were directed towards the alien threatened. The mandibles were held wide open. It was the position of the mandibles that was diagnostic of threat. The reaction of the worker was similar to the soldiers except that the mandibular opening was not as dramatic as

that of the soldiers. Threat, because it was an intention of attack, frequently preceded it and hence its classification as an aggressive response.

(4) Attack

Attack followed threat and in soldiers often seemed to be a continuation of threat. However, it was not uncommon to see a threat end abruptly by the slow closing of the mandibles. When attack followed threat the soldier closed its mandibles quickly and at the same time moved its body (not its legs) forward then backwards in the horizontal plane, at the intruder. This lunging, snapping movement inflicted severe injury to the alien. On a number of occasions it was found to have partly decapitated the alien termite. Attack by the workers was not as dramatic as that of soldiers. Threatening behaviour was followed by cautious approach by the resident towards the alien and this was followed by inflicting bites on it especially on the legs, antennae and abdomen. Extrusion of haemolymph as a result of the bites attracted other workers who attacked the alien simultaneously till the alien was totally immobilized. This was followed by the cannibalism of the individual or of 'walling' it up.

(5) Grooming

Grooming was often seen between nestmates in a normal colony (Chapter 3). Among other things grooming 'may facilitate the integration of the termite society'.

On the other hand aliens that gained entrance into the colony proper were rarely groomed and neither did they show any attempts to groom the residents. In order to establish if grooming was associated with hostility observations were carried out on (a) aliens that had gained entrance into the colonies, and (b) nestmates isolated for a day or for a fortnight. For purposes of comparison individuals within the nest, selected randomly, were also observed. The termites under study were observed continuously for thirty minutes. The results are summarised in Table 5.1.

It can be seen from the table that there was no significant difference in the frequency of grooming between individuals randomly selected within the nests and their nestmates isolated from them for one or fourteen days. But the aliens were subjected to very little grooming by the residents and the aliens in turn groomed the residents very little. (The difference in grooming was highly significant, $p > 0.01$). Thus in the case of the aliens it was seen that there was a negative relationship between grooming and aggressive behaviour. The few grooming bouts between residents and aliens always resulted in the biting of the aliens by the residents.

One of the interesting things observed was the fact that the highest grooming frequency (though not statistically significant) occurred with individuals returning to the nest after a day's isolation.

Table 5.1. The frequency of grooming in 30 minutes between residents and experimentals

Experimental Types	Frequency of grooming by experimentals		Time spent on grooming by experimentals		Significance (based on frequency)	Frequency of residents grooming experimentals		Time spent by residents grooming experimentals		Significance (based on frequency)
	Total	Mean	Total	Mean		Total	Mean	Total	Mean	
A Termites within nest	10	6.2	1569	156.9		79	7.9	1680	168.0	
B One day isolates	10	8.7	1539	153.9	A & B diff. not significant	94	9.4	1447	144.7	A & B diff. not significant
C 14 day isolates	10	5.5	1547	154.7	A & C diff. not significant	70	7.0	1581	158.1	A & C diff. not significant
D Aliens	10	3.1	474	47.4	A & D diff. highly significant $p > 0.01$	37	3.7	987	98.7	A & D diff. highly significant $p > 0.01$

This further strengthened the belief that nestmates were groomed as a matter of course and intruders were not. The increased grooming activity was probably due to the fact that foragers away from the nest probably 'adsorbed' adventitious odours onto their body surfaces. Differences in the odour were likely to evoke aggression and thus disrupt the integration of the colony. Grooming of the returned forager may be a way of incorporating the animal back into the colony by (a) removing the adventitious odour and (b) coating the forager's body with the salivary secretions of the colony. Thus it was social, as distinct from aggressive in function.

(6) Trophallaxy

Lange (1960) recorded a reduction in food exchange between individual ants of colonies fed on different diets. He used this discrimination as an indication of hostility between different communities. Similar techniques have been used by Montagner (1963 a, b) and Montagner and Courtois (1963) in vespine wasps. Wilson (1972) stated that this subtle distinction involving only rate of food exchange of aliens was first discovered by von Frisch and Rösch in their classical studies of the honey bees.

R. santonensis workers exchanged food freely amongst themselves within a colony. As reported earlier (Chapter 4) no distinction was made of nestmates isolated from the colony up to seventy-two hours. To study this further, i.e. to ascertain whether there was discrimination in food exchange between strangers and nestmates, an experiment was

done whereby the frequency of food exchange was measured between (a) individuals of a colony, (b) one and (c) fourteen day isolates and (d) aliens, in thirty minutes. Methods used to record this were similar to those reported in Chapter 4.3 , and the results are summarised in Table 5.2.

It can be seen from the table (5.2) that food exchange between nestmates and isolates showed no significant difference. On the other hand, during the period of observation food was seldom exchanged with aliens - the few exchanges seen were all proctodeal soliciting by the residents from the aliens.

Similarly to the observations on grooming, food exchange was carried out more frequently (though statistically not significant) with the one day isolates than with individuals within the nest and those isolated for two weeks. This suggested that individuals isolated for a day were treated as returned foragers and were often solicited for food or offered food by nestmates, and on the other hand, in the case of the fourteen day isolates food exchange was often inhibited initially on their return, but gradually restored as they 'lost' their adventitious odour adsorbed during isolation. To put it in another way, food exchange took place only after the individual had become integrated with the colony.

Thus it seemed that more grooming and trophallaxy and less aggression was shown towards nestmates and less grooming and trophallaxy and more aggression was shown

Table 5.2. Frequency of food exchange in 30 minutes between residents and experimentals

	EXPERIMENTAL	N	Frequency of food exchange		Bout length of exchange in sec.		Significance (based on frequency)
			Total Mean		Total Mean		
A	Individuals Within Nest	10	16	1.6	73	4.56	
B	Individuals isolated for 1 day	10	22	2.2	89	4.04	A & B diff. not significant
C	Individuals isolated for 14 days	10	8	0.8	31	3.87	A & C diff. not significant
D	Aliens	10	3	0.3	7	2.3	A & D diff. highly significant p > 0.001

towards aliens. This indicated that grooming and trophallaxy tended to re-incorporate nestmates into the colony but excluded aliens, thus helping to maintain the integration of the community.

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5.3. Factors Influencing Aggression

Besides odour other factors are also known to influence the hostility shown by social insects to members of the other communities. Some of the factors suggested by Wallis (1964) are visual and tactile, temperature, degree of hunger, movement, territory and presence of brood. Wilson (1966) stated that age of the colonies influenced aggression, i.e. incipient colonies were much more aggressive than mature ones. Nel (1968) in his study of H. mossambicus and Trinervitermes trinevoides (Sjöstedt) observed that visual, tactile and temperature influences did not play any major role but territory did.

5.3.1. Territory and Movement

Greaves (1962) recorded that in Coptotermes acinaciformis (Froggart) and C. brunneus (Gay) mature colonies were spread out over a large area and by implication this was due to intraspecific competition for the same territory. Nel (1968) in a series of experiments showed that in H. mossambicus aggression was not seen among termites using the same foraging areas though they were from different nests, but there was intense fighting between individuals not sharing the same area, when experimentally brought together. It was, of course, not possible to test territoriality in laboratory colonies of R. santonensis. However observations in plate nests showed that aliens discovered at the entrance of the main colony (normally entrance of Chamber A) were always attacked and injured, but when encountered in either Chamber B or C provoked alarm/flight responses rather than attack. Thus the experimental conditions in the plate were

such that the termites being observed showed aggression towards an alien termite only when entering Chamber A, thus entering their nest cell and hence their territory.

Movement of the alien was often found to increase aggression. Aliens at nest entrances, that remained stationary evoked only 'threat' from the residents. But if the alien moved the threatening resident lunged at the alien and injured it - the attack continuing till the alien was totally immobilized. Stuart (1969) remarked that a non-moving stimulus was of a lower intensity than a moving one. This seemed to be true in the case of R. santonensis.

Thus it can be seen that in at least R. santonensis the elicitation of aggressive behaviour may depend not only on odour but also on (a) the spatial position of the intruder and (b) its movement.

5.3.2. Brood

The presence of brood, by implication, also included the presence of the reproductive pair. Vowles (1952) and Schneirla (1958) suggested that presence of brood increased colony activity and was also an extra drain on colony resources, thus bringing about an extra need for food and perhaps an increase in hostility. Free (1955) correlated aggressiveness with ovary development and in honey bees Ribbands (1965) quoting Sakagami (1954) and Hoffman (1961) stated that queenless workers were more aggressive.

In order to ascertain the influence of brood on the aggressive behaviour of R. santonensis, the following experiment was conducted.

To measure hostility between individuals it was decided to use the four stages of aggressive behaviour described earlier, viz: 'examination', 'flight/alarm', 'threat' and 'attack'. These four stages were recognized as representing increasing stages of hostility. Sometimes it was difficult to distinguish between 'examination' and 'alarm/flight', since they tended to merge with one another, and so for purposes of analysis these two were treated as one. Just 'examination' and 'alarm/flight' alone were considered not to demonstrate hostility between two colonies.

A number of plate nests were set up from the same basic culture. Each nest contained two-hundred individuals made up of the different castes (as already described). After a few weeks two plate nests were selected one with eggs and young larvae and one without. Into these nests were introduced individuals from an alien colony one at a time, and the resulting behaviour was observed. The responses of the colony were observed by observing the introduced termite for thirty minutes; from the time it was encountered by residents. At the end of the thirty minute period the highest degree of hostility shown by the residents, to the aliens, was recorded. Not every individual encountered by the alien responded in a similar manner. Some residents totally ignored or were not aware of its presence while others reacted violently. The results of

Table 5.3. The effect of brood on the hostility of R. santonensis

Workers		No. and % of alien workers provoking							
From	To	Examination; Alarm/flight		Threat		Attack		Total	
		No	%	No	%	No	%	No	%
Colony X	Nest A (with brood)	5	25	5	25	10	50	20	100
Colony X	Nest B (without brood)	6	30	5	25	9	45	20	100

- (1) Nest A was a colony with brood and bred on Ilomba flour
- (2) Nest B was a colony without brood and bred on Ilomba flour
- (3) Workers from Colony X are individuals from a different community and bred on Ilomba flour

introductions of strange workers into the nest are tabulated in Table 5.3.

The results showed that R. santonensis colonies with or without brood showed hostility to strange workers. During the thirty minutes of observation, in 50% of the cases for nest A (with brood) and in 45% of the cases for nest B (without brood) the strangers were attacked*. However a χ^2 on the total response of the colonies indicated that there was no significant difference in their response to strangers ($\chi^2 = 0.143$, 2d.f., p, 0.95). If there was a difference in the aggression shown between the colonies with or without brood, then this was very small.

As a result of this study all subsequent studies were carried out in colonies, without regard to the presence or absence of brood and hostility was always shown to strangers in all studies.

5.3.3. Artificial Nests

It has been reported by Nel (1968) that laboratory colonies of H. mossambicus were less aggressive than field colonies. Similar reduction in hostility has also been reported for ants kept in artificial nests for long periods. As already explained, it was not possible to obtain fresh

*N.B. Subsequent examination of the plate nests later (after the 30 minute observation period) showed that a number of the strangers had been killed. No records of this were taken for analysis.

field colonies of R. santonensis for immediate experimentation in the laboratory. However the culture methods used for maintaining these termites indoors, very much represented field conditions, especially when one considers the fact that in its natural habitats Reticulitermes nests in pieces of damp wood, stumps, etc., and a colony may simultaneously occupy a large number of pieces of wood scattered over a wide area and interconnected by a network of galleries (Feytaud, 1912; Pickens, 1934). Since it was found that individuals of different cultures (colonies) showed hostility towards each other, it was decided to find if this hostility diminished as a result of keeping them in plate nests.

Termites were extracted from the two basic cultures (see Chapter 2) and set up in plate nests as described earlier. They were allowed to settle in the plates and observations were made on their aggressive behaviour by introducing individuals from one plate to another, one at a time. The introduced termite was kept under continuous observation for thirty minutes and the response it evoked from the residents was recorded for analysis. The results are summarised in Table 5.4.

In Table 5.4.

- (1) Workers from A are from colony A1 bred on Ilomba and are kept in the plate nests for less than two weeks.
- (2) Workers from B are from colony A2 bred on Ilomba and kept in the plate nests for less than two weeks.

Table 5.4. Effect of artificial nests on hostility of R. santonensis.

Workers			No. and % of workers provoking						Total	
From	To	Examination; alarm/flight		Threat		Attack				
			No.	%	No.	%	No.	%	No.	%
a	A	B	12	60	3	15	5	25	20	100
b	B	A	13	59.1	5	22.73	4	18.18	22	100
1	(a & b)		25	59.52	8	19.05	9	21.43	42	100
c	A ¹	B ¹	8	36.36	11	50.0	3	13.64	22	100
d	B ¹	A ¹	14	63.64	6	27.27	2	9.09	22	100
2	(c & d)		22	50	17	38.64	5	11.37	44	100
e	X	A ¹	5	25	7	35	8	40	20	100
f	X	B ¹	6	30	8	40	6	30	20	100
3	(e & f)		11	27.5	15	37.5	14	35.0	40	100

(3) Workers from A¹ and B¹ are the same as A and B except that they have been in the nests for longer than 4 weeks.

(4) Workers from X are individuals from an alien colony bred on Ilomba.

Using the X^2 test, it was found that,

(1) There was no significant difference in the aggressiveness shown in 1 and 2 ($X^2 = 4.5298$, 2df).

(2) There was significantly more hostility in 3 than in 1 ($X^2 = 8.6178$, $p > 0.02$, 2d.f.) and also more hostility in 3 than in 2 ($X^2 = 7.8813$, $p > 0.02$, 2d.f.).

The results therefore showed that though workers kept in artificial nests for four weeks or more showed less hostility, than those kept in the nest for less than two weeks; the differences between them was not significant. The aggression shown to aliens introduced into the plate nests after two weeks further indicated that conditions in artificial nests did not diminish the capabilities of the termite to distinguish aliens and attack them. Hostility was less between A and B than A and X and B and X. This may have been due to the fact that though individuals making up A and B were from different cultures they were basically bred on the same food and were from the same geographical area while workers from nest X were from a different geographical area.

5.3.4. Theories based on Odour

There has been a mass of evidence to suggest that in ants, honey bees and bumble bees recognition of members of a colony is by means of an odour distinct to the colony. In bees this colony odour has been recognized by such early naturalists as Bethe (1898) and von Buttel Reepon (1900) though experimental evidence for such a hypotheses was presented much later by Kalamus and Ribbands (1952) and Ribbands (1955) who demonstrated the existence of such odours in a series of experiments (a refinement of the 'Zwei-Völker-Versuche' procedures of Frisch and Rösch, 1926) showing that bees from the same colony were attracted to similar food sources and this attraction was basically due to similar odour. Further support for the role of odours in nestmate recognition was provided by Köhler (1955) who fed three adjacent colonies on sugar solutions flavoured with the same substance and found that many foragers took food from the other colonies and were not attacked by guard bees, but when the same colonies were fed with sugar solutions containing different flavours the robbers were severely attacked by the guard bees.

In ants, too, a similar system of recognition through odours was proposed as early as 1879 by McCook who was able to stop fighting between hostile colonies of Tetramorium caespium L. by introducing a pellet of eau-de-Cologne in their midst. Forel (1874) postulated something similar for the hostility shown by a number of ant species. Odour was implicated, again as a source of recognition by Formica fusca (Lubbock, 1894 and Wallis, 1962), by F. polyctena

Först (Lange, 1960, 1967) and by Myrmecia (Haskins and Haskins, 1950).

Free (1958) found that bumble bees recognized aliens through odour. He came to this conclusion when he found that workers of Bombus agrorum Fab. and B. lucorum L. stung anaesthetized alien workers introduced into their nests but not anaesthetized nestmates and moreover they attacked nestmates left in alien nests for two or three hours and then returned to their own nests.

Among termites non-nestmates were always recognized though this does not always result in fighting. Grassi and Sandias (1893-94) stated that K. flavicollis always recognized strangers and Andrews (1911) found that E. ripperti killed aliens. Recent work by Emerson (1929) on Nasutitermes, Pickens (1934) on R. hesperus and R. tibialis and Nel (1968) on Hodotermes and Trinervitermes all indicated that strangers introduced into their colonies were attacked. Emerson's (1929) investigation on the nest material and Andrews (1911) investigation on the water extracts of aliens and nestmates indicated that in termites, also, odour plays a key role in the recognition of nestmates.

In order to investigate the role of odour in the recognition of nestmates by R. santonensis, the following experiment was conducted. As already stated investigators of hymenopterans made use of different scented food material to develop different odours in the insects they studied. This was possible in honeybees and ants which fed on odorous material. For a long time it was believed that the

food of termites lacked any odour (Ribbands, 1965). But recent studies by Verron (1963), Smythe and Coppel (1966) and Moore (1969) among others showed that termite food does have odours (though not obviously discernable by humans) and that releaser pheromones that caused scent trails, defensive secretions, etc., are probably derived from food materials.

The Aston colonies from the Forest Products Research Laboratory were originally from the Saintes region in France (J. Taylor, personal communication). Two cultures from this original colony bred on Ilomba were obtained from the Forest Products Research Laboratory and these continued to be maintained on Ilomba, till early (March) 1972 when one of these colonies was subcultured and the new culture was maintained on spruce. Thus by the time hostility studies were conducted there were three colonies in the Aston laboratory, all originally from the same population but two of them fed on Ilomba and one on spruce.

A number of plate nests from each of the above colonies were set up. The wood flour that went into the plates being obtained by hammer-milling wood obtained from the respective tanks. This was done, so as not to upset any odour that might have been present in each of the cultures. As soon as the termites had settled themselves in Chamber C of the nests they were tested for aggressiveness. Nests that showed intense aggressive behaviour were selected for the experiment. Individual termites were removed from their own nests and introduced into one of the other two nests and vice-versa, to see if there was any difference shown

towards individuals fed on similar and different diets. In each case the results were obtained by observing the introduced termites continuously for 30 minutes and recording the response shown to it by the residents. The results obtained are tabulated in Table 5.5.

In Table 5.5.:

Workers from (a) A(il) and A¹(il) were individuals bred on ilomba and (b) workers from A(sp) were individuals bred on spruce for over fifteen months.

Using a X^2 test it was found that:

- (a) The hostility shown by (1) was greater than that shown by (2); ($X^2 = 5.06$, $p > 0.05$, 1 d.f.) (combining threat and attack as one cell)
- (b) (3) showed that there was no hostility shown to nest-mates isolated for one hour.

Therefore it was apparent that, in R. santonensis at least, difference in food and substrate (the Heterotermitinae ~~leaves~~, most often, lived inside their food) caused hostility between workers of the members of the same population. Since these differences cannot be genetic (as they were from the same original population) it must have been due to differences in odour.

Besides developing distinct odours by the metabolism of food, termites were also capable of 'adsorbing' adventitious odours in their cuticle (Emerson, 1929). Since the termites used in this study fed on and lived within the

Table 5.5. The effect of different foods on the hostility shown by workers of R. santonensis from the same population.

Workers			No. & percentage of workers provoking							
	From	To	Examination alarm/ flight		Threat		Attack		Total	
			No.	%	No.	%	No.	%	No.	%
1	A(il) A(sp)	A(sp) A(il)	5	22	5	22	13	57	23	101**
2	A(il) A ¹ (il)	A ¹ (il) A(il)	12	55	6	27	4	18	22	100
3*	A(il)	A(il)	8	80	2	20	0	0	10	100

* Control: nestmates isolated for one hour

** Rounding off of the results, does not add the percentages to 100.

same material it was not possible to distinguish between adsorbed and metabolised odour. Thus it can only be concluded that in the above experiment odour differences were produced as a result of feeding and living in different food materials.

5.3.5. The Origins of Colony Odour

The experiment in the previous section suggested that odour played a significant role in the identification of an individual as a nestmate or an intruder in R. santonensis. However the origin of the distinctive colony odour has been a subject of controversy. In as far as the Isoptera are concerned there has been little investigation to clarify this point. Verron (1963) based on his work on the inter-attraction of Kalotermes suggested both metabolic and genetical agents while Emerson (1929, 1939) and Stuart (1969, reporting an unpublished experiment) implicated environmental factors.

The question of the source of colony odour in Hymenoptera is a little clearer as a result of extensive experimentation by Lubbock (1894); Fields (1904); Brun (1912); Donisthorpe (1927); Haskins and Haskins (1950); Lange (1960, 1967) and Wallis (1962) on ants, von Frisch and Röscher (1926); Adam (1951); Butler and Free (1952); Kalamus and Ribbands (1952); Ribbands, Kalamus and Nixon (1952); Ribbands (1955); Le Comte (1952) and Chauvin (1968) on honeybees; Free (1958) on bumble bees and Rau (1930) and Montagner and Courtouis (1963) on wasps. Evidence gathered by these investigations implicated both environmental and

genetical factors in ants and bees and environmental factors alone in bumble bees.

Evidently the two possible sources of variation in colony odour viz: environmental and genetical are not mutually exclusive. Both could operate at the same time. The experiment described below attempted to test the roles of genetical and environmental factors in the establishment of odours in R. santonensis.

Experiment with R. santonensis

Plate cultures were set up as described in the previous experiment (5.3.4) from R. santonensis material from the following populations:-

- (1) The Aston cultures originally from Saintes, and
- (2)* The Centre for Overseas Pest Research cultures originally from La Rochelle.

Both these materials were collected between two periods separated by about eighteen months and from two centres about eighty miles apart. Though no attempt was made, in the present study, to establish the genetical differences of the above populations, it was not expected that they were of the same genetical material. Bouillon (1969) pointed out that populations of the same species separated or sufficiently isolated from one another may develop genetical differences as a result of the pressures of their

* Material obtained through the kind generosity of Mr. R.M.C. Williams of C.O.P.R.

different habitats.

As soon as the termites had settled in Chamber C of their plate nests, individuals were removed from their own nests and introduced into one of the other two nests (made of termites of the same population but on a different food and termites from another population but on a similar food) to see if there was any difference shown towards them. Observations were carried out for thirty minutes and the response was recorded for analysis. The results are tabulated in Table 5.6.

In table 5.6.:

1. A(il) refers to workers from the Aston culture bred on ilomba and originally from Saintes.
2. A(sp) refers to workers from the Aston culture bred on spruce and originally from Saintes.
3. X(il) refers to workers from the Centre for Overseas Pest Research bred on ilomba and originally from La Rochelle.
4. X(sp) refers to workers from the Centre for Overseas Pest Research bred on spruce and originally from La Rochelle.

Using the χ^2 test it was found that

- (a) there was no significant difference in the hostility between 1 and 2 ($\chi^2 = 2.76$, 2d.f.)

Table 5.6. Aggression shown towards workers from genetically different communities

	Workers		No & percentage of workers provoking							
	From	To	Examination alarm/ flight		Threat		Attack			
			No.	%	No.	%	No.	%	No.	%
1	A(il)	A(sp)	11	38	8	28	10	34	29	100
	A(sp)	A(il)								
2	A(il)	X(il)	6	19	11	35	15	47	32	101*
	X(il)	A(il)								
3	A(il)	X(sp)	5	16	6	19	21	67	32	102*
	X(sp)	A(il)								

* exceeds 100% as a result of rounding.

- (b) there was no significant difference in the hostility between 2 and 3 ($X^2 = 2.9$, 2d.f.), and
- (c) there was a significant difference in the hostility between 1 and 3 ($X^2 = 6.28$, $p > 0.05$, 2d.f.).

The results therefore indicated that great hostility was shown towards genetically different populations even though they have been bred on the same food. However the hostility shown to the individuals of the alien population (on identical food) was not very much greater than that shown to individuals from the same population but bred on different food.

Thus the results of the above investigation showed that in R. santonensis the mechanism of colony odour was to a great extent influenced by genetical factors but that these factors were modified by local environmental conditions like different food, nest material, etc. This was further confirmed by the fact that in the experiment described above individuals from alien populations on dissimilar food showed significantly more hostility than members of the same population on dissimilar food.

5.4. Discussion and Conclusion

The results of the study described in this chapter clearly showed that R. santonensis was able to distinguish between individuals of their own and other colonies. The response to intruders of alien colonies varied with the different individuals of the colony. This was probably why not all the intruders were attacked and killed in the experiments conducted. The responses could sometimes include overt acceptance of the alien into the colony but with little grooming and very little trophallaxis. Since observations were limited to just thirty minutes only during this study, there was no evidence as to the ultimate fate of those intruders that successfully gained entrance. But the fact that nestmates isolated for 14 days and reintroduced into the colony were gradually integrated into the colony may suggest that those aliens which are not violently rejected might eventually be accepted as a result of their acquiring the colony 'odour'. The experiments of Emerson (1929) with Nasutitermes may be relevant here.

Many factors contributed to the degree of aggression shown to the intruder. The situation at which intruders were discovered constituted a major factor. Alien termites discovered, especially by soldiers, at nest entrances, were vulnerable to attack. Wallis (1964) reported that in F. fusca, an ant appearing at a nest constituted a situation which alerted the guard ants and might evoke aggression in them. There is no evidence in literature to suggest that there are guard workers among R. santonensis. But soldiers of the colony were normally found at nest entrances and

galleries, and Howse (1970) considered their behavioural thresholds to stimuli were lower in such situations, than in the open especially when they are pointing in the right direction (i.e. towards the alien). It is worthwhile to consider here if the situation of the experimentals could have contributed to the variable results obtained by Andrews (1911) and Nel (1968). The former mixed different colonies in finger bowls to test their interaction and the latter used metal pans. Such experimental situations can make it (a) difficult to distinguish alien odour and (b) increase the threshold of the stimuli in the open thus giving uncertain results. Wilson (1971) stated that social insect workers generally lost most or all of their hostility when removed from their nest. Therefore it was most important that in all studies involving hostility to strangers adequate precautions were taken before experiments were performed.

Ambient temperature and humidity conditions were considered important in the provocation of aggression in the hymenoptera (Wallis, 1964). Since subterranean termites, including Reticulitermes are capable of controlling the microclimate of their environment, it was doubtful if these factors were important at all. Nel (1968) did not consider temperature as an important factor in the aggressiveness of H. mossambicus. The presence of brood and breeding in artificial nests did not affect the aggressiveness of R. santonensis in the above studies. Nel (op cit) also found brood to be of little importance. However, he found that laboratory colonies were less aggressive than field colonies.

Finally the results obtained in the experiments described, indicated that odour was an important factor in the provocation of hostility. While genetically inherited factors seemed to play an important role in the determination of nest odour, environmental factors also played an important part in the process. The hostility shown to an alien population on similar diet was no greater than that shown to members of the same population on a different diet. This hypothesis was further strengthened by the investigations of Emerson (1929), showing that the epicuticle of the termite was capable of adsorbing nest odour from nest material, Andrews (1911) found that individuals which had been washed were attacked by nestmates.

Thus it seems probable that, in R. santonensis at least, colony odour was formed by the combined odours of food, nest material and inherited factors.

CHAPTER VI

CHAPTER VICANNIBALISTIC AND NECROPHAGIC BEHAVIOUR6.1. Introduction

Cannibalism and necrophagy are very pervasive among termites. It ranges from the elimination of supernumerary reproductives by K. flavicollis (Lüscher, 1952; Rupli, 1969) to the ritualised cannibalism practised by the primary reproductives of some African species (Grasse, 1942). Williams (1959) recorded that in Cubitermes ugandensis (Fuller) the primary reproductives after the construction of their nest cell, but before copulation, eat up to five articles of the terminal segments of each others antennae; later they consumed some of their eggs and young larvae of their filial brood. Ratcliffe and Gay et al (1952) reported that in almost all of the Australian termites they had investigated the general tendency was to eat all their weak and injured, both in laboratory and natural colonies. They further stated that among Coptotermes lacteus Holmgren the imagoes that failed to swarm were eventually killed and eaten by the workers.

The reasons for this pervasive cannibalism among termites are still uncertain. As evidenced by the work of Lüscher (1952) and Rupli (1969) it was a factor in the determination of castes. Other than that Cook and Scott (1933), Hendee (1935) and Andrew (1930) reported that cannibalism was a means of increasing the amount of protein available in the colony. On the other hand Ratcliffe and

Gay et al (op cit) did not think that protein conservation was the primary reason for cannibalism, but nest sanitation was.

Besides cannibalism of injured and weak individuals and supernumerary reproductives, almost all cadavers were also disposed of by necrophagy or by storing them in middens. Gay and Calaby (1970) reported that the Amitermes laurensis Mjöberg stored their dead in middens and Bouillon (1970) stated that the Trinervitermes bettonianus Sjöstedt stored their dead in the mounds. Both species used their stored cadavers as food.

Besides termites, none of the other social insects ever eat their dead except workers of Solenopsis saevissima which do eat them under laboratory conditions (Wilson, 1971). The usual response, of the social hymenopterans, was to discard their dead in rubbish heaps. However oophagy and brood cannibalism was fairly common especially in times of food deprivation.

Cannibalism and necrophagy among R. santonensis has been observed by Buchli (1950) and Williams (personal communication). Alien workers killed in combat were devoured while their wounds were still fresh (Chapter 5). In order to elucidate the factors governing these (cannibalism and necrophagy) phenomena further, the work described below was undertaken.

6.2. Confirmation of Cannibalism and Necrophagy in R. santonensis.

Since the first essential basis for the study was that R. santonensis should show cannibalism and necrophagy of its own nestmates, it was decided to confirm this by:

(1) Selecting individual workers at random from the plate cultures and injuring their abdomen and amputating their appendages and returning them to their plate nests

As a result of doing this the following behaviour was observed.

The injured individuals did not attract attention immediately. However, cannibalism of the injured termite started gradually, beginning with the examination of the injury by the workers and soldiers. This was followed by the gradual licking and nibbling of the injured spot ultimately leading to chewing of the injured worker often at the abdominal and thoracic regions. Complete consumption of the injured termite took up to two days, in some cases.

(2) To confirm necrophagy, individual workers were selected at random from the colony and these were killed by freezing them (in the freezer compartment of an ordinary domestic refrigerator). The cadavers were then introduced one at a time into the plate nests. The following behaviour was observed.

Attention to the cadaver was shown almost as soon as it was discovered. The individual that discovered the

cadaver either examined the corpse by pointing its antenna towards it or more usually by palpating the corpse with its antenna. When no response was forthcoming from the cadaver, the 'examiner' sometimes showed an 'alarm/flight' reaction or nibbled the corpse. The corpse was sometimes dragged about in the colony and eventually it was fed upon by chewing by one or a number of workers. This often went on for hours. When a number of workers were involved in the necrophagic activity the cadaver was often pulled apart. There was, however, no special sequence in the feeding on the corpse. Andrews (1930) reported that in Termopsis augusticollis the intestinal tract was often the first to be consumed. This was not so in R. santonensis.

(3) Not all cadavers in the colony were necrophaged. Some of the dead individuals were 'walled up'. This behaviour was observed by killing the termite in a hot ($>80^{\circ}\text{C}$) oven overnight and introducing them into the plates one at a time the following morning.

The components of behaviour involved in the 'walling up' process was essentially the same as necrophagic behaviour except that after dragging the corpse for some time it was dumped in a corner or cul-de-sac of the plate where it often remained unattended for some time. When 'walling up' began the sequence of activity was similar to that of building (see Chapter 3.3). The termites went through the series of activities of fetching and carrying wood particles and cementing them with faecal or oral cement around the corpses, till they completely entombed them. This sequence of activity was not rigid and termites involved in the

'walling up' process were involved in other activities as well, but eventually came back to the cadaver until the entombing was complete.

It showed that the primary reason

was the primary reason

was colonies of Zootermopsis.

6.3. Some Factors Influencing Cannibalism in *R. santonensis*

The investigations of Andrew (1930), Cook and Scott (1933) and Hendee (1935) all showed that insufficient diet or the lack of protein, especially, was the primary reason for cannibalism in normal laboratory colonies of Zootermopsis (Termopsis) augusticollis.

Termites derive their main source of energy and metabolic water from cellulose (wood). Cellulose, free from fungal or bacterial infection has a very low amount of nitrogen and termites cultured in pure cellulose often make very slow growth (Hungate, 1941). It has been suggested, that in order to make up for this small amount of nitrogen in their food most termite species practised controlled cannibalism by eating eggs, young larvae, supernumerary reproducives, injured, dying, dead and the exuviae (Moore, 1969). To investigate if a shortage of nitrogen or protein in the food of *R. santonensis* provoked cannibalism the experiment described below was done.

6.3.1. Influence of diet on Cannibalism

Plate nests were set up each containing 100 individuals as described in Chapter 2.4. As the study was carried out to study the influence of diet, three different types of food were used. The plates contained the following material.

- (1) Ilomba wood flour from rotting wood taken from the stock cultures.

- (2) Ilomba wood flour from seasoned wood that looked 'apparently' free from fungal infection, and
- (3) Pure cellulose (Whatman filter paper No. 1). The filter paper was shredded into small fibres and this was changed daily to prevent the growth of any micro-organisms.

A fourth set of plates, containing just sterile vermiculite was used to study the effects of total starvation on cannibalism.

The nitrogen content of the two types of (1 and 2) wood flour used was analysed using a micro-kjeldahel technique (Humphries, 1956), while that of the Whatman filter paper was obtained from the manufacturers. This (the nitrogen content) data is tabulated in Table 6.1.

Table 6.1. The percentage nitrogen found in the diet used to study cannibalism in *R. santoneñsis*.

Diet	No. Samples used	mean % nitrogen
1 Infected Ilomba	20	0.166%
2 'Uninfected' Ilomba	20	0.049%
3 Filter paper	-	0.010%*
4 Vermiculite	-	0.

* manufacturers figures

Five plate nests for each type of diet were set up and the termites introduced into them. Observations were made almost daily and records were made of all individuals attacked and cannibalised. The observations were carried out for six weeks, at the end of which the plate nests were dismantled and a count made of all the termites remaining alive. A count was also made of all the uneaten head capsules and also the uneaten corpses (especially in the plates containing just vermiculite, where due to the general weakening of the colony no effort was made either to necrophage or wall up cadavers). The observational data and the actual count gave an accurate account of those individuals that were cannibalised. Table 6.2 gives the number of individuals that were cannibalised.

Table 6.2. The influence of diet on cannibalism

Replicate	Number Cannibalised			
	Infected Ilomba	Uninfected Ilomba	Filter Paper	Vermiculite
1	2	4	8	18
2	3	5	9	15
3	2	6	11	15
4	1	3	12	16
5	3	4	10	18
Total	11	22	50	82
n	5	5	5	5
\bar{x}	2.2	4.4	10.0	16.4

variance ratio: 127.53, p. ≤ 0.001

The results clearly showed that the quality of the diet influenced the amount of cannibalism among R. santoniensis ($p > 0.001$). As one would expect the greatest amount of cannibalism occurred in the colonies that were completely deprived of food. With increasing starvation there was an increase in the interaction (grooming, soliciting) between individuals. This often resulted in minor injuries which attracted other individuals and eventually resulted in the consumption of the injured.

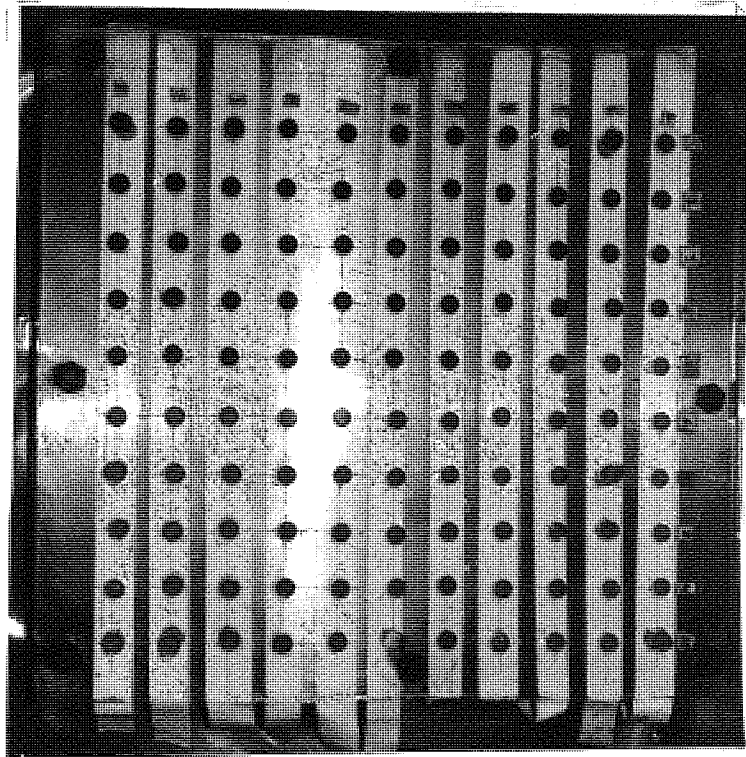
On the whole these results confirmed those obtained for Z. augusticollis by earlier investigators. It is not known if starvation of other substances besides nitrogen would have any influence on cannibalism.

As injury seemed often to result in cannibalism its influence was next investigated.

6.3.2. The influence of injury on cannibalism.

Not all injuries that occurred in a colony resulted in cannibalism. It was not uncommon to observe, in healthy colonies, individuals with one antenna or a leg missing and still continuing to be active in grooming, trophallaxis and other activities in the society. However, severe injuries always resulted in the elimination of the injured animal. In order to investigate if there was a threshold level of injury at which cannibalism commenced, a preliminary experiment was carried out to establish an injury index. A number of termites from the plate cultures were removed and these were surgically mutilated (by the removal of one antenna,

Plate 6.1. A battery of isolation chambers in perspex



puncturing the abdomen, etc.) and isolated from the rest of the colony for forty-eight hours in tiny isolation chambers bored out of a perspex sheet (33 x 30cm; Plate 6.1). The chamber was half-filled with wood flour and kept moist. The mouth of the isolation chamber was closed with a cover slip to prevent the termite from escaping. At the end of the forty-eight hour period, the mortality rate of the injured (isolated) termites was taken. Uninjured termites were kept isolated and these acted as controls. The result is given in Table 6.3.

Table 6.3. The effect of different levels of injury on the mortality of individual termites.

	No. presented	No. Dead at 48hr	% Dead
A Amputation of all legs and antennae	10	10	100
B Amputation of all legs and puncturing of abdomen	10	10	100
C Amputation of all antennae and puncturing of abdomen	10	10	100
D Amputation of all legs only	10	10	100
E Puncturing of the abdomen only	10	9	90
F Amputation of all antennae only	10	6	60
G Amputation of part of the antennae and legs	10	4	40
H Amputation of part of one leg	10	3	30
I Amputation of part of one antenna only	10	1	10
J Control (isolated but not injured)	10	0	0

As the results, in Table 5.3, show, termites subjected to severe mutilation obviously died, while those suffering minor mutilation survived during the period of isolation. From these results an injury index was arbitrarily established using the following criteria:

- Injury index 1: where less than 20% of those injured died
 Injury index 2: where between 20-40% of those injured died
 Injury index 3: where between 40-60% of those injured died
 Injury index 4: where between 60-100% of those injured died.

Sixteen plate nests were set up as described earlier (using infected ilomba flour as substrate/food). Twenty four individuals were selected from each plate and these were surgically injured (to fall into one of the four injury indices). After injuring them the injured animals were returned to their respective colonies. The colonies were periodically examined for a week at the end of which the number surviving was recorded. The results are tabulated in Table 6.4.

Table 6.4. Number of surgically injured termites surviving in plate colonies

Replicate	No. surgically injured	Number surviving			
		Injury Index			
		1	2	3	4
1	24	24	23	17	1
2	24	23	21	13	0
3	24	23	24	13	2
4	24	24	20	10	0
Total		94	88	53	3
\bar{x}		23.5	22	13.25	0.75
S.dev.		± 0.5	± 1.5	± 2.5	± 0.8

variance ratio: 136.37, $p > 0.001$

The results clearly indicated that the level of injury was an important factor in the ultimate fate of the injured termite in healthy colonies. Severely injured individuals, especially those with abdomens punctured or crushed, had a continuous extrusion of body fluids. This extrusion of the body fluids constantly attracted the attention of colony mates which licked and eventually bit those areas that were injured. In the case of individuals having minor injuries (like a broken leg or antenna) attention was shown to them, initially, by colony mates, but in due course healing of the wound, manifested by the blackening of the injured area occurred. This blackening, caused probably by the precipitation of quinones (Williams, 1957) often repelled colony mates from locking or biting the 'healed' area.

Thus it seemed that the presence of fresh haemolymph (or components present in the haemolymph) often acted as a releaser of cannibalism. This was further investigated by dabbing filter paper discs with haemolymph and presenting them to termites, as described below.

6.3.3. The role of haemolymph in cannibalism

An attempt was made to extract haemolymph from termite workers. This was undertaken by collecting about one thousand termites from stock cultures. Part of the antennae and legs of these termites were snipped off so that there was a flow of haemolymph from the injury. The injured termites were then placed in a modified miniature centrifuge

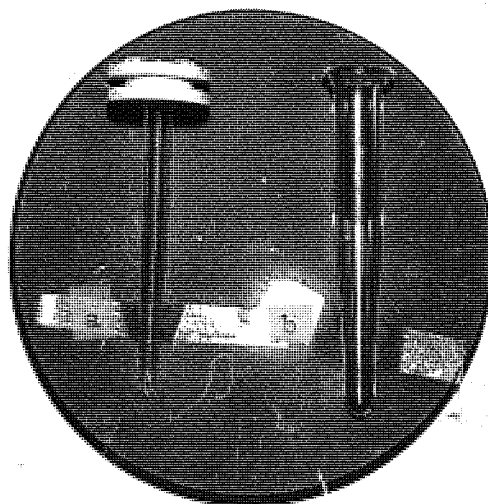
tube with a minute ($< .025\text{mm}$ diameter) hole at one end. The termites were placed on top of a layer of glass wool. The 'termite containing tube' was then placed into another centrifuge tube which acted as a jacket. The jacket did not have any hole in it (Plt. 6.2). This assembly was then packed tightly (with cotton wool) into a centrifuge flask. This was then centrifuged under refrigeration (at -15°C) at about 1000 rpm for thirty minutes. The centrifuging extracted most of the haemolymph present in the termites; this was collected in the jacket.

Using a thin glass rod the haemolymph was dabbed on to filter paper discs (5mm in diameter). Filter paper discs dabbed with distilled water were used as controls. Treated and control discs were presented to termite colonies simultaneously and their response over twenty-four hours was recorded for analysis. The results are presented in Table 6.5.

Table 6.5. Response to haemolymph-treated filter paper discs.

TREATED				CONTROL			
Eaten		Uneaten		Eaten		Uneaten	
No.	%	No.	%	No.	%	No.	%
16	80	4	20	5	25	15	75
15	75	5	25	6	30	14	70
14	70	6	30	6	30	14	70
17	85	3	15	4	20	16	80
18	90	2	10	3	15	17	85

Plate 6.2. Apparatus used for extracting haemolymph from termites.



- a - tube holding termites
- b - jacket for collecting haemo-lymph

The results showed clearly that haemolymph or some component present in the haemolymph acted as a releaser for cannibalism. A χ^2 carried out on the results showed that the difference in the response to treated and control discs was highly significant ($\chi^2 = 60.5$, $p > 0.001$, 1df).

A few conclusions may now be arrived at from the experiments described:

- (1) In laboratory colonies of R. santonensis cannibalism seemed to be triggered off by the need for protein,
- (2) while an injury was often the beginning of cannibalism, minor injuries in healthy colonies received little more than normal attention, but severe injuries (especially when the injured animal was no longer able to carry out the social activities of the colony) resulted in cannibalism. In this respect the removal of the individual from the colony can also serve a sanitary purpose (for an injury that does not heal can be a source of pathogenic infection (Wilson, 1971)), and
- (3) The releaser of cannibalistic behaviour, seemed to be either the haemolymph or compounds present in the haemolymph.

6.4. Factors Influencing Necrophagic Behaviour

Most ants carry their dead nestmates, stereotypically to rubbish dumps where they discard them. Wilson, Durlach et al (1958) and later Blum (1970) reported that chemical releasers, probably a fatty acid or a combination of fatty acids, were responsible for this necrophoric behaviour.

In normal healthy colonies of R. santonensis, the freshly dead are mostly necrophaged. However not all cadavers were treated likewise. Some cadavers were covered up with wood fragments, defaecatory material, etc., i.e. they were 'walled up'. This 'walling up' of cadavers often happened when they were badly decomposed or died as a result of "unnatural causes". Gay and Calaby (1970) and Bouillon (1970) respectively suggested that Amitermes laurensis Mjöberg Trinervitermes bettonianus stored their dead in middens for use as food. The 'walling up' of cadavers in laboratory colonies of R. santonensis did not so much indicate food storage operations but rather a means of 'shutting off' an alarm causing substance from their midst. Experiments described below were carried out to investigate the factors that influenced necrophagy among R. santonensis in the laboratory.

6.4.1. Response to Termites Killed by Various Methods

Preliminary experiments indicated that termites killed in an oven ($> 80^{\circ}\text{C}$) under five minutes were generally necrophaged while those left in the same oven for more than two hours were 'walled up'. This suggested that the causes of

death might have a bearing on the ultimate fate of the cadaver.

Plate cultures were set up as described earlier. As soon as the termite workers had settled into the plates, 'workers' were extracted, from the stock colonies from which the termites for the plate cultures had been extracted and these were killed by six different methods. A total of sixteen plate cultures were used for this study and into each plate twelve cadavers, per treatment, were introduced. The cadavers were left in the plates, one at a time and up to four termites were introduced on any one day. The results are tabulated in Table 6.5.

Table 6.5. Response to cadavers killed by different treatments

	Treatments	No. of Repl.	Observed frequency of necrophagy	Total Nec.	Mean Nec.
1	Heat (≤ 5 min.)	16	10, 8, 9, 10, 11, 10, 9, 10, 12, 10, 11, 11, 10, 12, 9, 11	163	10.19
2	Heat (> 120 min.)	16	2, 3, 1, 2, 1, 2, 1, 1, 0, 2, 1, 1, 2, 3, 0, 0	21	1.31
3	Freezing	16	11, 12, 12, 10, 10, 12, 12, 12, 12, 12, 12, 12, 9, 12, 10, 11	181	11.31
4	Crushing	16	12, 12, 12, 12, 10, 12, 10, 11, 12, 12, 11, 12, 9, 12, 10, 11	180	11.25
5	Chloroform	16	0, 0, 2, 1, 1, 0, 0, 1, 0, 0, 1, 2, 2, 0, 0, 0	10	0.63
6	Carbon Tetrachloride	16	2, 1, 0, 0, 0, 0, 0, 1, 2, 1, 1, 1, 2, 0, 0, 1	12	0.75
	TOTALS			568	35.57

variance ratio: $f = 604.48$, $p > 0.001$

The results clearly showed that the difference in response to the different treatments was highly significant ($p > 0.001$). While it was obvious why termites killed with fumigants, having a strong odour, were treated with alarm and thus disposed of by 'walling', the reasons for the unpalatability of termites killed as a result of intense heat was not clear.

To investigate the possibility that the termites might be deterred from necrophaging termites killed by intense heat because of their dryness, the bodies of the under-five-minute heat killed and the over-two-hours heat killed cadavers were thoroughly damped by spraying their bodies with distilled water. They were then offered to the colonies in the same manner as earlier tests. The results are presented in Table 6.6.

Table 6.6. Response to termites killed by two levels of heat and damped

Total No. Presented	Heat \leq 5 min.		Heat $>$ 2 hour	
	Necrophaged	Walled Up	Necrophaged	Walled Up
12	9	1	3	6
12	8	2	2	5
12	10	1	1	7
12	11	0	0	8
12	10	1	1	6
12	12	0	1	6
Total	60	5	7	38

A χ^2 on the results showed that the preference to necrophage the under-five-minute cadavers was significantly higher than those killed in heat for over two hours ($\chi^2 = 65.89$, $p > 0.001$). Thus it seemed conclusive that the dampness or succulence of the cadaver had little to do with the response. It seemed probable that intense heat might have altered the chemical composition of the cadaver and made it unattractive to the termites.

6.4.2. Response to Cadavers of Different Castes

Individuals of the four different castes used during this entire study were collected from stock cultures and these were killed by freezing. The cadavers were then presented to the colonies in the plate nests one at a time. The response of the colonies were recorded and this is presented in Table 6.7.

Table 6.7. Response to Cadavers of Different Castes.

N = necrophagy W = walling up

No. presented	Workers		Replacement Reproductives		Soldiers		Larvae	
	N	W	N	W	N	W	N	W
12	9	0	9	1	7	2	9	1
12	8	2	10	0	6	1	8	0
12	9	3	8	1	6	2	8	1
12	10	1	9	0	7	0	10	1
12	12	0	7	0	8	0	11	0
12	10	2	8	0	9	0	10	2
Total	48	8	51	2	43	5	56	5

No statistical analysis was done on the results as it was obvious that no discrimination was shown between cadavers of the different castes.

6.4.3. Response to Cadavers of Alien Colonies and Alien Species.

The termite nest and its immediate environment offers shelter to several organisms. While some of these organisms treat the termitarium only as a shelter (the termitariophiles) others carry an obligatory relationship with the termites (the termitophiles) (Kistner, 1969). Besides the termitariophiles and the termitophiles, termites also come into contact with other species of termites and other species of insects (e.g.) ants; while termitariophiles and termitophiles are tolerated within the colonies, others are promptly killed. In order to find if cadavers of other species were necrophaged by R. santonensis the experiment described below was carried out.

Individual insects of various species were killed by freezing and these were then presented to the plate colonies. The response of the colonies is tabulated in Table 6.8.

No analysis of the data was carried out as it was fairly clear that only cadavers of the same species were necrophaged, while those of other species were walled up.

Table 6.8. Response to Cadavers of different species

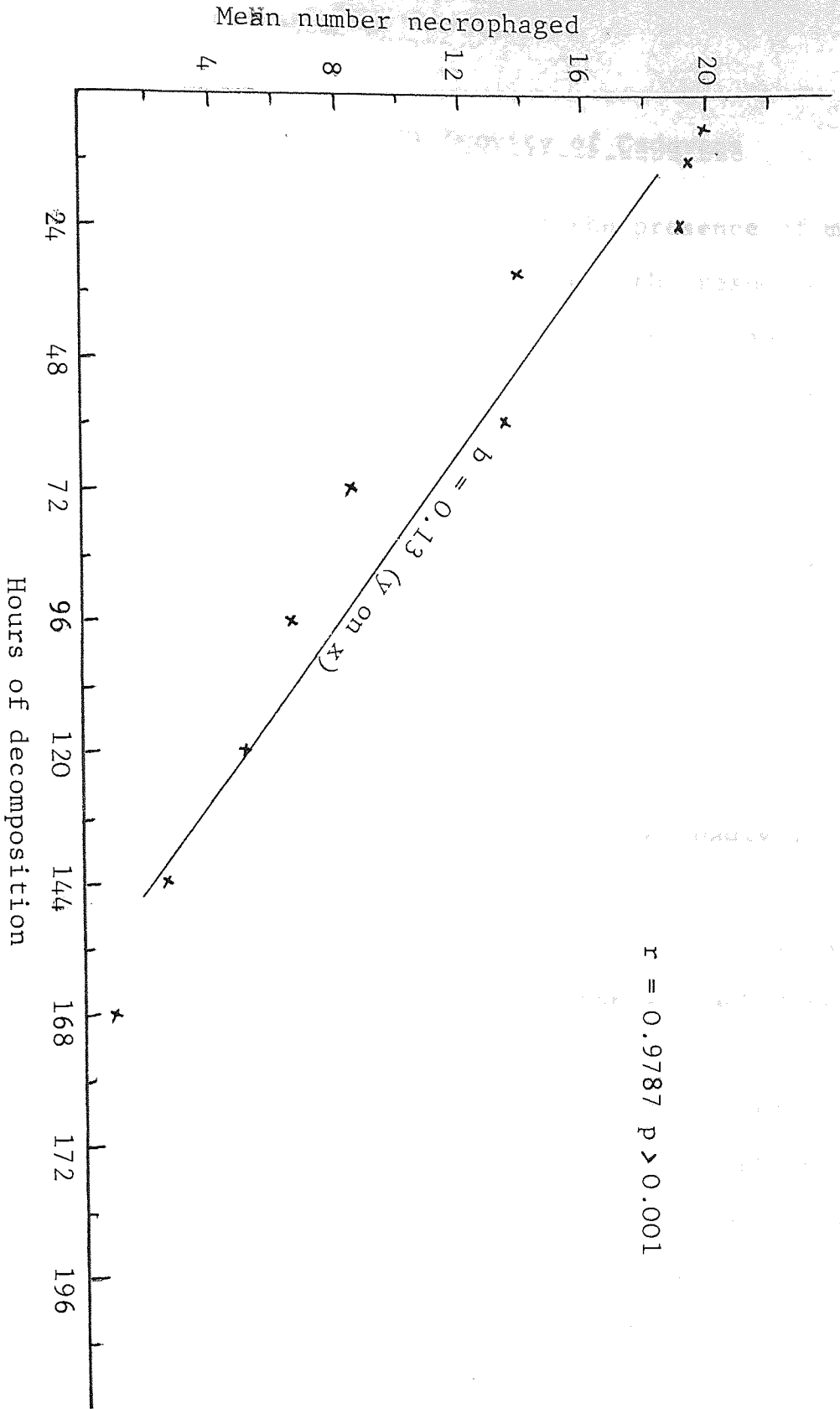
	Cadavers	No. presented	Necrophaged	Walled Up
1	R. santonensis (same colony)	48	39	3
2	R. santonensis (alien colony)	48	38	5
3	R. lucifugus	48	2	32
4	Z. nevadensis	48	0	34
5	Staphalynids (sp?)	28	0	19
6	Ants (sp?)	48	0	39

6.4.4. Response to Decomposing Cadavers

It was noticed, during the course of experiments already described, that cadavers left to decompose for longer periods tended to be walled up or were not eaten. It was thus decided to establish if the state of decomposition of the cadaver determined the response shown to it. Randomly selected individuals from plate cultures were killed by freezing them. The cadavers thus killed were allowed to decompose in the open laboratory for varying periods of time. These were then offered to the colonies in plate cultures and their response recorded.

The results (of individuals necrophaged) are presented graphically in Fig. 6.1., which shows a significantly negative correlation ($r = -0.9787$, $p. > 0.001$). Less than 50% of the cadavers were necrophaged after eighty-four hours of decomposition and less than 10% of the cadavers were con-

Fig. 6.1. Response to decomposing cadavers.



$r = 0.9787$ $p > 0.001$

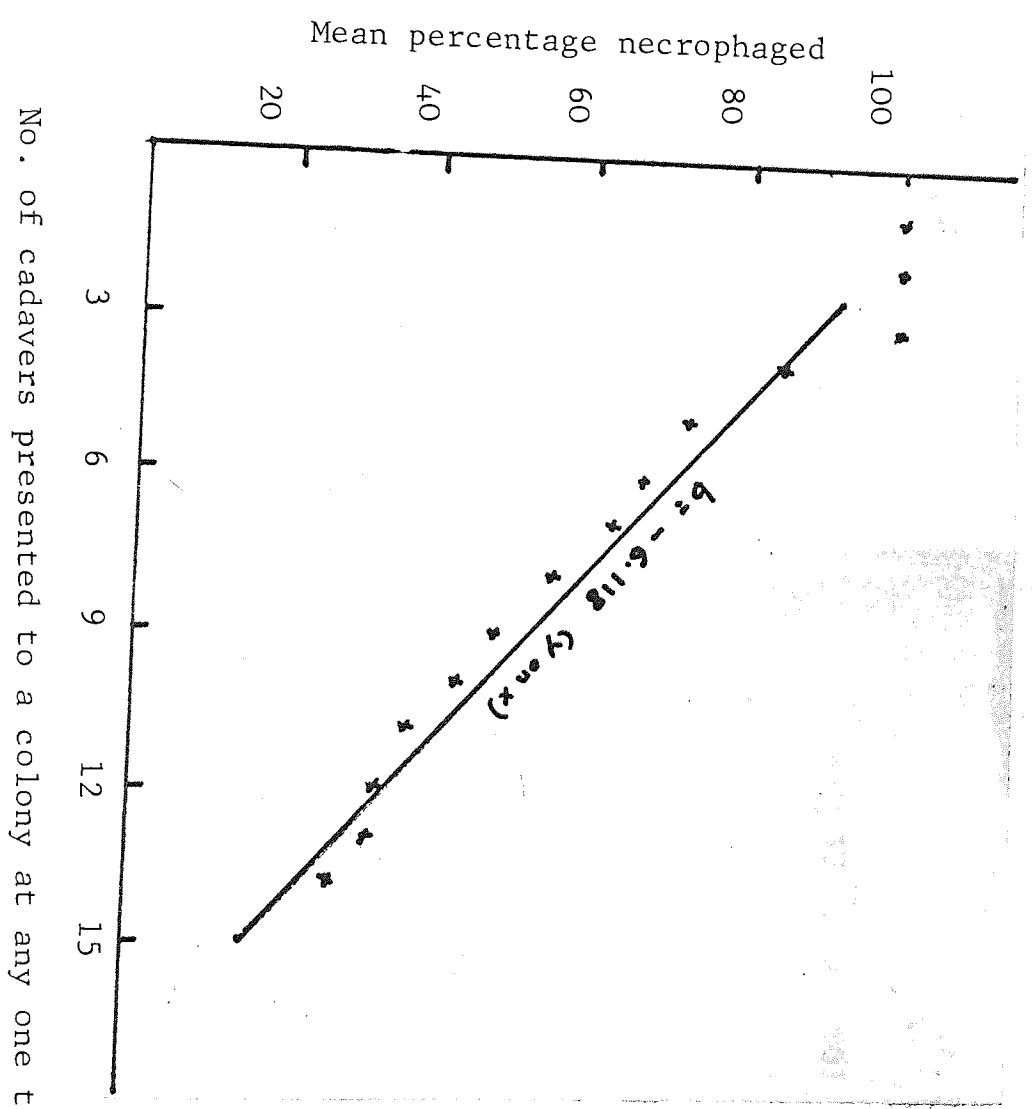
sumed after 144 hours (6 days). Almost all uneaten cadavers were 'walled up'.

6.4.5. Response to the Density of Cadavers

An attempt was made to find if the presence of multiple cadavers simultaneously, influenced the response of the colony. This was done by freeze killing randomly selected individuals and presenting them to the plate colonies lot by lot. In order to eliminate the building up of sequential association between the presentations, the four replicates were divided into two groups. The first group was presented with cadavers starting with one corpse and increasing the number with each lot, while the second group was presented with twenty cadavers to start and gradually reduced to one. Each lot was presented as soon as the unnecrophaged cadavers started to decompose 'badly'.

The results were first analysed for sequential association and it was found that the response was not different between the two groups. Hence the results of both the groups were pooled and this was examined for correlation between density of cadavers and percentage necrophaged (Fig. 6.2.). The regression line drawn shows that there was a negative correlation between density and necrophagy ($r = -0.98$, $p > 0.001$) thus indicating that R. santonen-
sis colonies were able to consume only a certain number of cadavers at any one time and those that were not eaten decomposed badly and hence were 'walled up'.

Fig. 6.2. Response to density of cadavers presented simultaneously to a colony



From the above series of experiments some conclusions can be drawn.

- (1) R. santonensis colonies recognized and thus necrophaged cadavers of their own species, while those of alien species were not necrophaged.
- (2) Cadavers of all castes were necrophaged, but individuals that died as a result of 'unnatural' causes were found to be unpalatable, and similarly those that died as a result of intense heat. Badly decomposing cadavers were also not necrophaged. Since cadavers of all types lack any movement it thus seemed probable that termites distinguished cadavers of the various categories by olfactory stimulus.

Thus all the experiments pointed to the fact that, like ants, termites 'recognized' death through chemicals that 'signalled' death. While some 'signals' (those recognized) released necrophagic behaviour 'strange signals' caused alarm. Alarm causing materials were, stereotypically, walled off.

To investigate the role of chemicals further, an attempt was made to isolate any possible 'death' pheromones.

6.4.6. Chemical Releasers of Necrophagy

A search for the chemicals responsible for releasing necrophagic behaviour was made by the assay of decomposition products of R. santonensis bodies. This was done by removing workers from the stock cultures and killing them by

freezing. In order to remove any chemicals that may be present on the body surface, crude extractives of the cadavers were made. This was done with (1) distilled water and (2) acetone and both freshly killed individuals and cadavers decomposed for 96 hours were used for the extraction. The resulting cadavers were then presented to the colonies in plate nests. Besides presenting the cadavers, filter paper discs (5mm in diameter) treated with the extractives were also presented to the plate nests. Less than 5% of the extracted cadavers elicited any response at all, showing clearly that the compound 'releasing' necrophagy was removed from the cadaver. However, when filter paper discs treated with the extractive were presented to the colony it too elicited very little response. Less than 2% of the 60 discs presented provoked any response at all and these were necrophaged. The rest were mostly ignored. The only possible explanation that can be offered for the failure of the filter paper discs to elicit response was that the extractive procedures adopted were (1) crude and (2) the amount of material used for extraction was too small (3) some components of the extractive could have been lost during extraction.

Since fatty acids present in the bodies of ants were implicated in the necrophoric behaviour of ants (Blum, 1970), it was thought that fatty acids found in termite bodies might provoke necrophagic or walling up behaviour.

To test this, an attempt was made to put on filter paper discs a range of fatty acids found on termites.

Carter and Dinus et al (1972) had identified more than twenty-five compounds but the major fatty acids found were oleic (58.6%), linoleic (10.4%), palmitic (10.2%), palmitoleic (3.2%) and stearic (5.5%) acids in R. flavipes. The above fatty acids along with a few more and some protein decomposition products obtained as pure chemical reagents were made up in three concentrations (using acetone or distilled water as solvents) giving 1%, 10% and 100% of the compounds. Using an "Agl" micrometer syringe filter paper discs 5mm in diameter were treated to give an equivalent of 0.01 μ g; 0.1 μ g and 1 μ g of the material per disc. The treated discs and controls (treated with acetone and distilled water) were presented to the colonies. The results are tabulated in Table 6.9.

The results obtained were not statistically analysed, as the aim of the experiment was to locate broadly substances that might act as releasers. From the ratio of necrophagy/walling up it was seen that the three major fatty acids (1, 2, 3) found in termites provoked necrophagic behaviour at the lowest concentrations butyric acid, stearic acid and caproic acid were mildly active. The rest of the fatty acids and all the protein decomposition products were found to cause alarm. At the medium concentrations once again the first four fatty acids were mildly active in inducing necrophagy but the rest tended to cause alarm and finally, at the highest level of concentration, all the materials tested caused alarm rather than necrophagy.

Table 6.9. Response to filter paper discs treated with fat and protein decomposition products.

N = necrophagy

W = walling up

	Chemicals	Response at <i>behaviour</i>								
		0.01 ug			0.1 ug			1 ug		
		N	W	Ratio of N:W	N	W	Ratio of N:W	N	W	Ratio of N:W
1	Oleic acid	29	2	14:5:1	19	22	0.86:1	1	28	0.04:1
2	Linoleic acid	28	3	9.33:1	13	16	0.81:1	1	29	0.03:1
3	Palmitic acid	27	3	9:1	15	19	0.79:1	1	30	0.03:1
4	Butyric acid	21	4	5.25:1	9	19	0.47:1	0	32	0:1
5	Caproic acid	9	9	1:1	3	25	0.12:1	0	33	0:1
6	Caprylic acid	9	18	0.5:1	6	30	0.2:1	0	35	0:1
7	Valeric acid	3	12	0.25:1	3	24	0.13:1	2	30	0.03:1
8	Lauric acid	4	32	0.12:1	0	34	0:1	0	35	0:1
9	Stearic acid	12	12	1:1	6	24	0.25:1	0	35	0:1
10	Formic acid	6	9	0.67:1	0	35	0.1	0	36	0:1
11	Phenylethylamine	0	34	0:1	0	32	0:1	0	36	0:1
12	Triethanolamine	0	35	0:1	0	36	0:1	0	36	0:1
13	Ammonium Sulphide	0	30	0:1	0	33	0:1	0	35	0:1
14	Phenol	0	29	0:1	0	28	0:1	0	30	0:1
*	Acetone (1 to 9)	4	7	0.57:1						
*	Distilled Water (10 to 14)	8	2	4:1						

* Controls

From this preliminary study some conclusions may be drawn, viz:

- (1) R. santonensis used a limited array of chemicals in recognizing cadavers.
- (2) The chemicals that released necrophagic behaviour seemed to be the fatty acids which are found extensively in insect bodies. Whether any of the other chemicals present in bodies of termites released necrophagic behaviour is not known, and
- (3) All chemicals that were not fatty acids were treated with alarm and walled up.

6.5. Discussion and Conclusion

In natural colonies of termites the interiors of termitariums were kept free of injured, dying and dead nestmates. Several authors (Ratcliffe and Gay et al, 1952; Bouillon, 1970) have shown that this was done through cannibalism of the injured and dying and necrophagy of the dead. This process of eliminating the dying and the dead helped to supplement the little protein that was available to the colony in predominantly cellulosic diets (Moore, 1969).

Observations on laboratory colonies of R. santonensis showed that neither all injured nestmates nor all dead nestmates were consumed. Many of the mildly injured functioned normally within a colony; and often unpalatable cadavers were shut off from the rest of the colony by 'walling up'. These observations were similar to those of Williams (personal communication) with his colonies of R. santonensis.

The results of the experiments described in Section 6.3.1, confirmed the results obtained by Andrew (1930) Hendee (1935); Cook and Scott (1933) that protein starvation increased the amount of cannibalism in laboratory colonies. With decreasing amount of protein or food there was often an increase in the grooming, soliciting activity between individuals in a colony. This seemed to result in an increased number of minor injuries, which in a colony already starving often resulted in the elimination of the injured. While minor injuries in under nourished colonies led to cannibalism, in 'well fed' colonies this was not found to be so. It was found that in colonies having adequate nourishment

only injuries that resulted in the inability of the injured to carry out the functions of the society resulted in elimination of the injured individuals. This it seemed that the response to injury was governed by the level of injury and the stress the colony was under. It was also found that minor injuries healed faster and ^{quinones} ~~quinones~~ or ~~ran-~~ related chemicals found in the area of the healing wound acted as mild repellants. Injuries that did not heal rapidly acted as a source of attraction and it seemed probable that haemolymph or some chemical compound or compounds in the haemolymph acted as a releaser to cannibalise. This was confirmed by experiments (6.3.3) on filter paper discs treated with haemolymph which were attacked and consumed as soon as it was left in the colony.

While injury undoubtedly attracted attention through haemolymph (or chemicals present in it), it seemed surprising that death was also signalled by chemicals. The experiments carried out pointed to the fact that the response was to a narrow range of chemicals found in decomposing bodies. The fact that only cadavers of the same species were necrophaged while those of others were 'walled off' indicated that this range of chemicals might be governed by a species factor.

Further experiments indicated that pheromones were responsible for 'signalling' death and the fate of the cadaver depended on the type of 'signals' it gave. More (1969) recognized that releaser pheromones mediate most short term interaction between individuals including the

disposal of the injured and dead. Wilson, Durlach et al (1959) and Blum (1970) had already demonstrated the role of pheromones in the disposal of the dead among ants.

Attempts made to extract the chemicals were successful in removing the necrophagic response but attempts to transfer the response to filter paper discs were not very successful. This failure was probably due to the crude extractive procedures adopted. However the fact that a few filter paper discs treated with extractives were all eaten points to the fact that given suitable equipment it would probably be possible to analyse the extractive and determine its chemical nature.

Based on the experience of Wilson and Durlach et al (op cit) on hymenoptera; fatty acids and protein decomposition products were exposed to R. santonensis on filter paper discs. The results showed that fatty acids like oleic, linoleic, palmitic all released necrophagic behaviour at low concentrations. Most of the other chemicals tested evoked alarm.

Thus it seemed that R. santonensis colonies utilized a relatively limited array of chemicals in recognizing their injured and dead. While haemolymph or some compounds present in it triggered cannibalism of the injured, the disposal of the dead was brought about by fatty acids accumulating in the bodies of cadavers and released either through autolytic catabolism which accompanies necrobiosis or the hydrolytic activity of bacteria that might be found in the dead bodies.

CHAPTER VII

1. The first of these is the

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CHAPTER VIITHE INFLUENCE OF ARSENIC TRIOXIDE
POISONING ON TERMITE BEHAVIOUR7.1. Introduction

Social insects, in general, have developed effective means to integrate the functions of their colonies. This cohesiveness appears to be maintained through the utilization of a series of communicative elements in their behaviour. Wilson (1971) has listed the modes of communication found in social insects and pointed out that most communication systems appeared (especially among termites) to be based on chemical singals.

This seemed to be so, at least in R. santonensis, which were found to utilize an array of olfactory stimuli in the recognition of nestmates, exchange of food and disposal of their injured and dead. Moore (1969) suggested that chemicals were also implicated in the determination of castes, trail laying, recruitment, alarm, etc., in almost all termite species.

Since experiments, carried out during the course of this study, showed that both living and dead termites employed chemical stimuli for social regulation; it brought forward the hypothesis that colonies would be able to distinguish nestmates 'exposed' to toxicants and thus prevent them from contaminating the whole colony. Observations reported by Randall and Goody (1934), on the ability of dampwood

termites to protect their colonies from arsenic contamination, by sealing runways and galleries dusted with that poison seemed to indicate that such a phenomena can happen. In order to test the validity of the hypotheses, on R. santonensis, individual termite workers, extracted from plate cultures, and treated with Arsenic trioxide, were observed on their return to the plates and the responses of their nestmates were recorded for analysis.

7.2. The Toxicity of Arsenic Trioxide

Arsenic, in its many forms, has been used as a termiticide, from as early as 1916 (Randall and Doody, 1934) and though it has not found much success against subterranean termites (especially in the U.S.A.) it is still being used in the Australian and S.E. Asian region. In S.E. Asia the toxicant is used as a 30% concentrate in either a graphite or talc base. The dust is applied by means of a blower through breaches made either in the runways of termites or in woodwork at 18-24 inch intervals.

In order to establish the toxicity of arsenic trioxide a bioassay was done using termite workers weighing between 1.5 and 2.5mg. These were made to run through a glass tube eighteen inches long and having a bore of 2mm. The internal wall of the tube was dusted with arsenic trioxide in graphite by means of a 'Kaligun' blower. This left a thin coating of the dust on the walls of the glass tube and termites running through the tube 'picked up' the poison. Preliminary experiments using coloured dust showed that in running through the glass tube the experimentals were picking up dust particles on all their appendages and on some parts of their dorsal and ventral abdomen.

Five concentrations of arsenic trioxide were used in graphite giving 0.005, 0.05, 0.5, 5 and 50% (w/w). Termites made to run through tubes containing unadulterated graphite were used as controls. Treated and control termites were isolated in individual isolation chambers (Plate 6.1.).

The results of the bioassay are presented in Table 7.1.

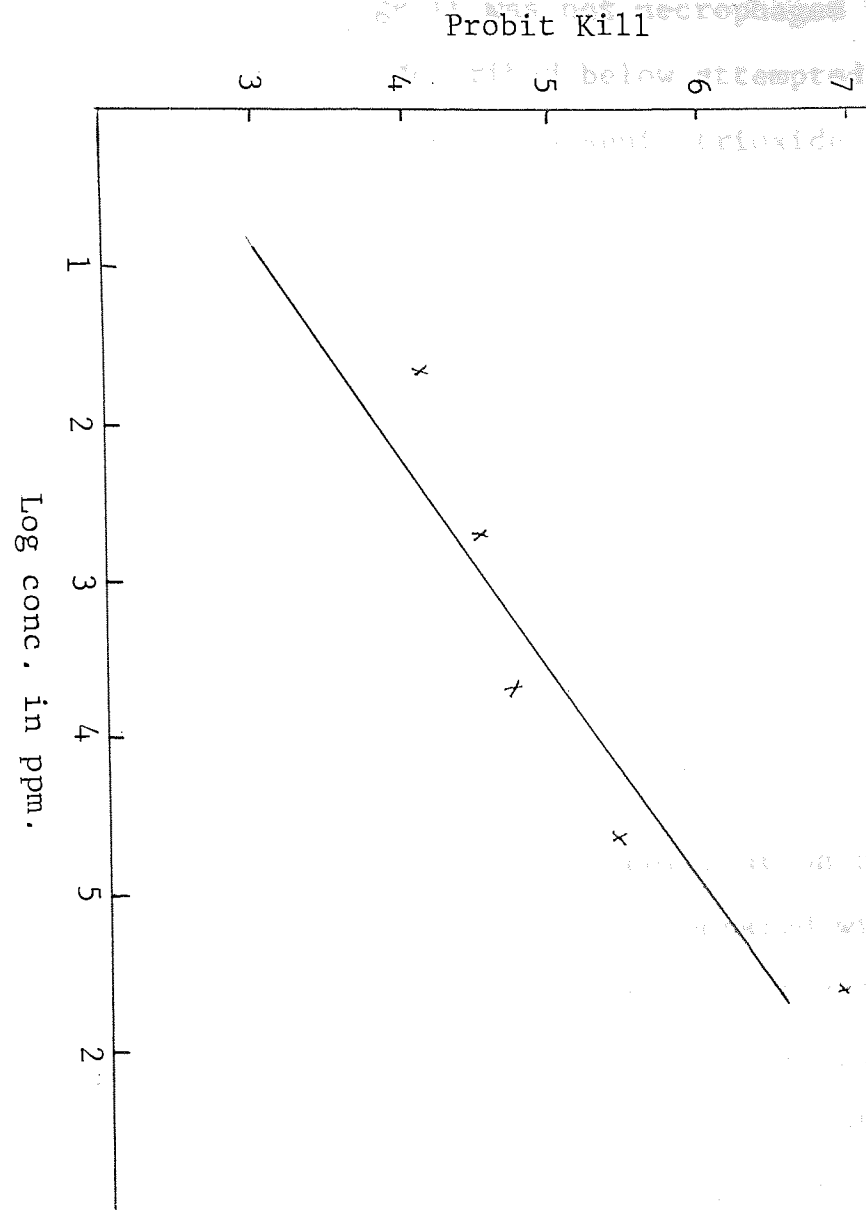
Table 7.1. The toxicity of AS_2O_3 to R. santonensis (72 hours after exposure).

Conc. of AS_2O_3 mg/g.	No. of termites	No. killed	% killed	Probit kill	Corrected* probit
500	100	98	98	7.05	6.68
50	100	69	69	5.5	5.41
5	100	44	44	4.85	4.72
0.5	100	36	36	4.64	4.5
0.05	100	24	24	4.29	4.08
0	30	2	6.67		

* N.B. - using Abbots formula

A regression line fitted by eye is presented in Fig. 7.1. This gave the LD_{50} for AS_2O_3 as 2.2 mg/g (or 0.22%). However this figure should be treated with caution as toxicity is the result of the actual amount picked up by each individual insect and the method used could not ensure precisely equal pick up. Attempts to measure the actual amount picked up by individual termites by chemical assay were not successful.

Fig. 7.1. The relation between probit of kill of *R. santonensis* and concentration of arsenic trioxide in graphite (regression line fitted by eye)



7.3. Response to Cadavers, Killed by AS_2O_3

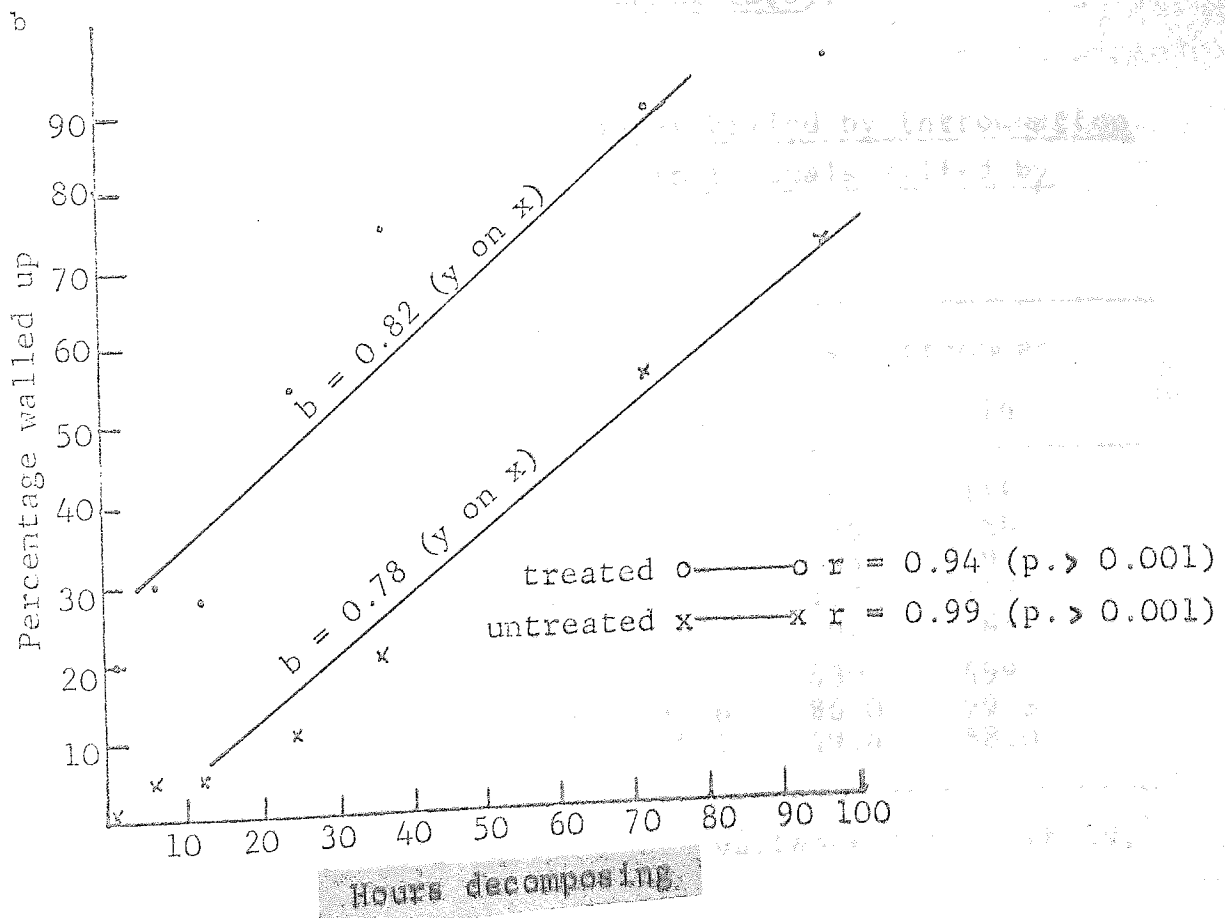
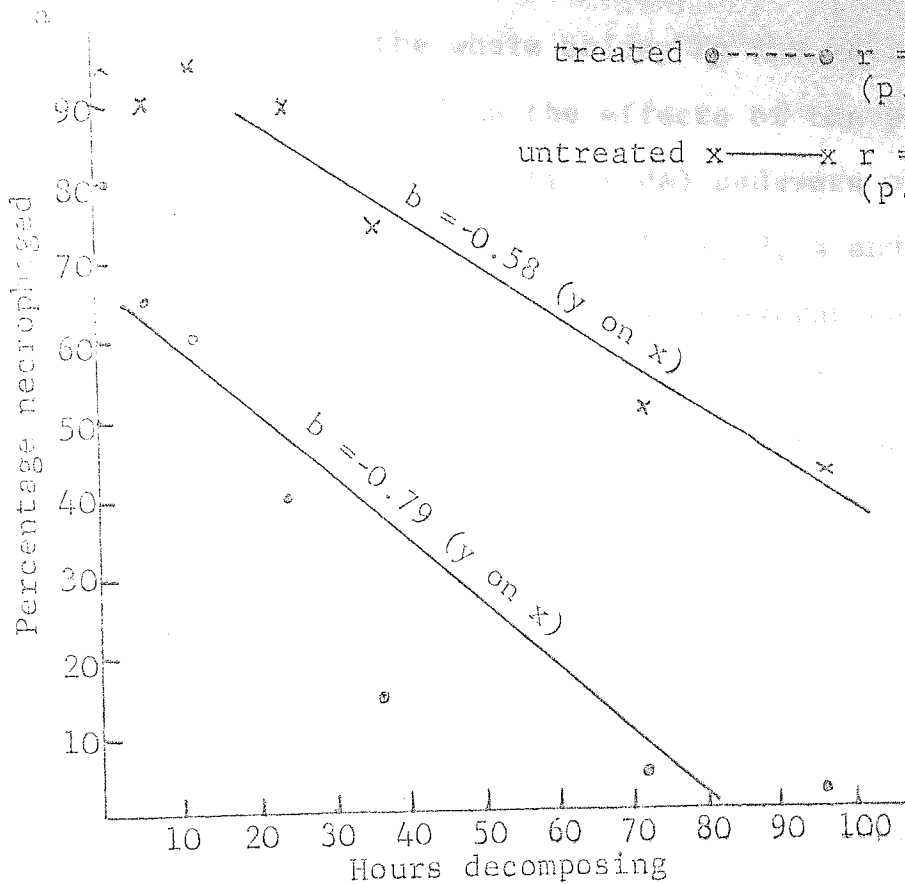
The normal response to a freshly dead nestmate in a colony was to necrophage it. However if a cadaver presented stimuli that were strange it was not necrophaged but was walled up. Experiments described below attempted to analyse the response of colony mates to arsenic trioxide killed individuals.

Worker termites were selected randomly from plate nests and these were made to run through glass tubes dusted with 30% AS_2O_3 in graphite. On emergence from the glass tubes they were kept in isolation till they died. Cadavers were then allowed to decompose for varying lengths of time and presented to the colonies. The responses of the nestmates were recorded for analysis.

The results were examined for correlation between decomposition and response. This was compared with the response to cadavers that died through freezing (Data obtained from Chapter 6.4.4.). The results (Fig. 7.2a and b) clearly show that (1) untreated cadavers were attractive for necrophaging for a significantly longer time than the treated ones. Less than 50% of the treated cadavers were eaten after twenty hours decomposition whereas more than 50% of the untreated were eaten even after eight-four hours decomposition.

Conversely within a day more than 50% of the treated cadavers were walled up whereas 50% of the untreated ones were only walled up after seventy hours decomposition.

Fig. 7.2. The effect of decomposition in (a) necrophagy and (b) walling up between treated (poisoned) and untreated cadavers.



Since the arsenic trioxide cadavers were presented to the colony one at a time they did not or 'were prevented' from contaminating the whole colony by the walling up process. In order to find the effects of the presence of freshly killed (by arsenic trioxide) cadavers on a colony an experiment was done wherein 0.5, 1, 2, 4 and 8% of members of plate colonies of two-hundred were extracted, poisoned and returned to their colonies on dying. The colonies were then kept under observation for a week. At the end of the observation period, the whole plate colonies were dismantled and an account was taken of those living, dead and necrophaged, dead and walled up and dead and left unattended (i.e. neither necrophaged nor walled up).

Table 7.2 gives the total number of nestmates killed as a result of contamination (sensus lato).

Table 7.2. The number* of nestmates killed by introduction of various numbers of individuals killed by arsenic trioxide

Replicate	No. of AS ₂ O-killed cadavers introduced				
	1	2	4	8	16
1	0	14	26	67	118
2	0	17	80	46	88
3	9	47	48	93	97
4	10	59	74	117	107
5	9	51	60	107	89
Total	28	188	288	430	499
\bar{X} killed	5.6	37.6	57.6	86.0	99.8
% killed	2.8	18.9	29.4	49.4	58.0

* excluding individuals introduced. variance ratio: 18.39,
p > 0.001.

The results clearly showed that the total effect on the colony was increased in proportion to the logarithm of the number of poisoned cadavers introduced into the colony and the relationship appeared to be linear (see Fig. 7.3). However, in the plate colonies even though up to 60% of the individuals of colonies were killed, the rest were still alive after a week and an examination of the living indicated that none of the dependant castes like soldiers and replacement reproductives were affected by the poisoning.

An analysis of the dead showed that some were necrophaged, some walled up and some unattended. The results were examined for correlation between response of the colony and the total killed. This is presented graphically in Fig. 7.4a, b and c.

Fig. 7.4a shows that there was no correlation between the number killed and number eaten ($r = 0.14$). Obviously not all the dead were eaten and neither was there any proportional increase in the number eaten to the number dead. Thus it seemed that sometimes only a few (presumably the freshly dead) were necrophaged while the rest were treated with alarm and uneaten.

But an examination of Fig. 7.4b did not show a positive correlation between the total dead and number walled up. On the contrary there was a significantly negative correlation ($r = -0.55$; $p, 0.001$). Evidently the colony was successful in walling up all the uneaten dead at lower levels. However with increasing death and consequently

Fig. 7.3. The relation between varying numbers of (introduced) poisoned cadavers and nestmates killed

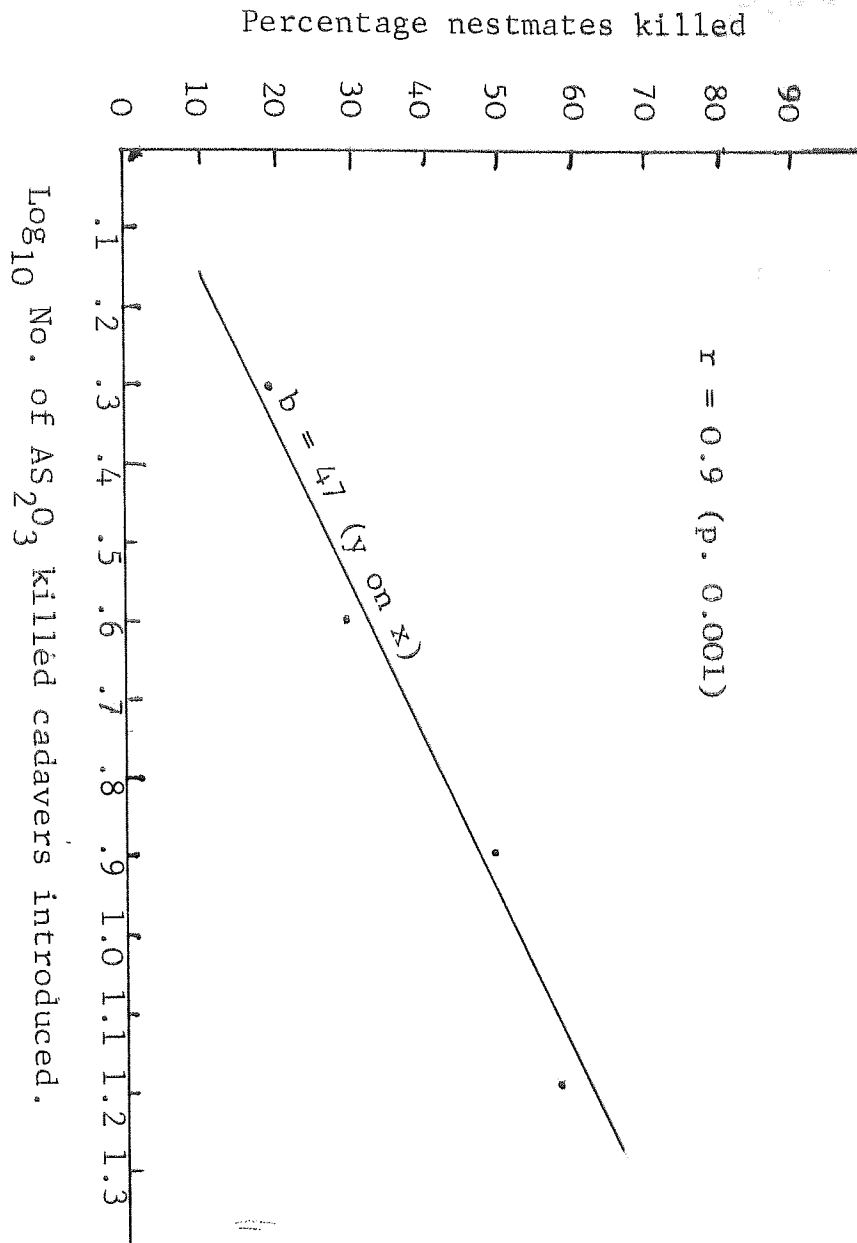
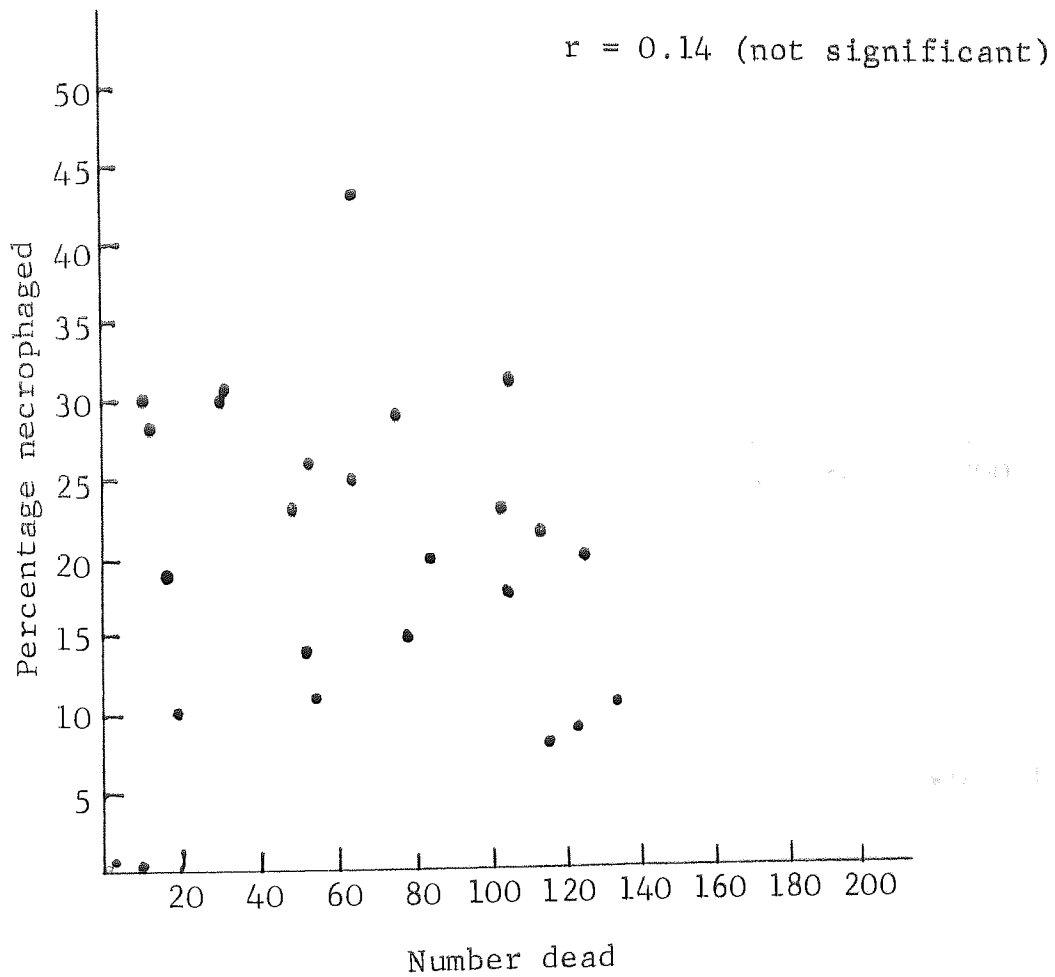
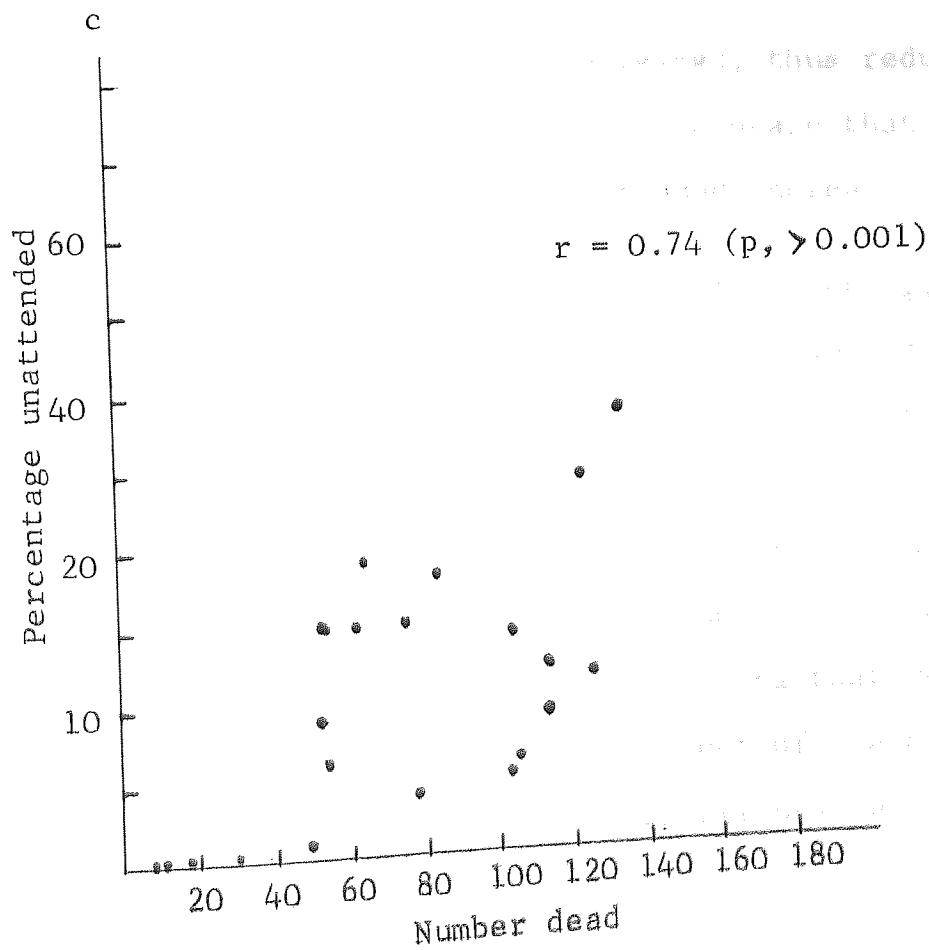
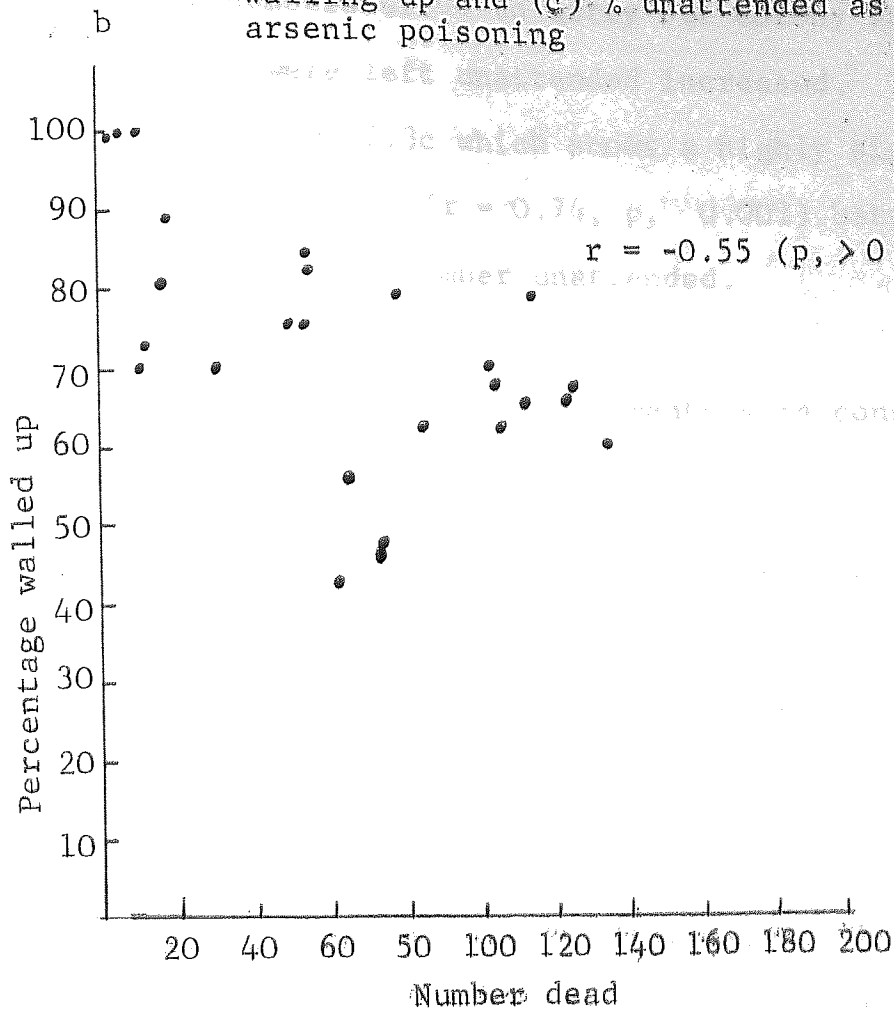


Fig. 7.4a. Correlation between total killed and percentage necrophaged as a result of arsenic poisoning



7.4. (b&c) The correlation between total dead and (b) % walling up and (c) % unattended as a result of arsenic poisoning



decreasing numbers alive to carry out colony function - the numbers that were left unattended increased. This is clearly indicated in Fig. 7.3c which shows a highly significant positive correlation ($r = 0.74$, $p, 0.001$) between the number dead and the number unattended.

Thus from the above experiments some conclusions can be drawn:

- (1) Living R. santonensis nestmates were not able to discriminate between freshly dead individuals that were treated with arsenic trioxide and those that were not. However they were able to distinguish between decomposing and fresh cadavers.
- (2) While freshly dead were necrophaged, the decomposing cadavers were walled up, as long as the total dead was small. However when the total dying increased, thus reducing the remaining living population, the percentage that was walled up dropped while those left unattended increased.
- (3) Even though, at higher levels of poisoning, half the nest population died, those that survived were found to be healthy and capable of carrying out colony functions.

However it must be pointed out that the introduction of a toxicant through a cadaver was unnatural - as in field conditions it was the contaminated living that were expected to carry the poisonous dust to the rest of the colony. In order to examine this situation experiments described below were conducted.

7.4. Response to Nestmates Carrying AS_2O_3

Termite workers were extracted from plate cultures and these were forced to run through glass tubes treated with ^{30%} AS_2O_3 . As soon as the termites had emerged out of the tube they were returned to their plate nests and the responses (grooming and trophallaxis) of their nestmates were recorded for analysis. The results are tabulated in Table 7.3.

Table 7.3. Response of *R. santonensis* to nestmates treated with AS_2O_3 .

Experimental	n	mean frequency of grooming	mean sig- nificance 't' test	frequency of Troph- allaxis	signific- ance 't' test
*1 Termites within nest	10	9.4		1.6	
*2 Termites isolated for 1 day	10	7.9	2 & 3 not sig.	2.2	2 & 3 not sig.
3 Termites carrying AS_2O_3	10	8.8	1 & 3 not sig.	2	1 & 3 not sig.

* Data from Table 5.1 and 5.2.

It can be seen from the table that no distinction was made by nestmates between untreated and those returning with arsenic trioxide. Thus it seemed probable that a termite contaminated with arsenic trioxide would be capable of transmitting the poison to the rest of the colony on its return.

In order to establish the effect on nestmates of varying numbers of poisoned individuals returning an experiment was conducted whereby 0.5, 1, 2, 4 and 8 percent of the total population of two-hundred were extracted from plate nests and these were made to run through AS_2O_3 treated glass tubes. Immediately on emergence from the tubes they were returned to their nests. The nests were kept under observation for a week and at the end of the period the colonies were dismantled and an account was taken of the living, the dead and necrophaged, the dead and walled up and the dead and left unattended. Table 7.4 gives the result of the total number killed as a result of contamination.

Table 7.4. Number* of dead termites in colonies of 200 as result of introducing nestmates treated with AS_2O_3 .

Replicate	Number carrying AS_2O_3 into plate nests				
	1=0.5%	2=1%	4=2%	8=4%	16=8%
1	6	66	121	118	164
2	5	72	142	146	164
3	7	80	74	180	168
4	30	2	137	157	177
5	26	87	106	156	176
Total	74	307	580	757	849
mean killed	14.8	61.4	116	151.4	169.8
% dead**	7.4	31.0	60.0	78.8	92.3
Effectiveness ⁺	14.8	30.7	29.0	18.9	10.6

Variance ratio: 39.49 p. > 0.001

* excluding the introduced individuals

** % of 1000 termites (less those carrying AS_2O_3) in 5 nests.

+ Effectiveness = number of nestmates killed per treated individual introduced.

The results clearly indicate that the total number of individuals killed in the colony was governed by the number of termites returning with the poison. Since all the carriers of the toxicants were alive at the time of their return they were thus able to transmit the toxic material to nestmates through grooming (initially) and through trophallaxis (secondarily) and possibly by arsine gas. Thus by the end of the week in colonies receiving 0.5% poisoned individuals there was a mean mortality of about 7.5%; 1% - > 25%; 2% - > 50%; 4% - > 75% and 8% - > 90%. Analysis of the results showed that the difference in the mortality was highly significant. Examination of the surviving individuals indicated that in all the colonies the dependant castes were all alive at the time of examination.

Further analysis of the data was carried out to find out the number of nestmates killed by each returning individual treated with arsenic trioxide. This was found to rise to 30 when the proportion treated was between 1 and 2%. It is a remarkable fact the effectiveness of the lowest number of dosed individuals (1 per plate, 0.5%) is less than the next two higher numbers. It would be expected that a single returned individual would receive at least as much attention in the way of grooming and trophallaxis as each of the two or four individuals. This may be positive evidence for the effect of arsine since the amount of this gas produced in a single plate colony would probably be proportional to the number of dosed individuals and may be below a threshold of toxicity at the lowest level.

The results were also examined for correlation between the total number killed and the ultimate fate of the cadavers. These are graphically presented in Fig. 7.5 a, b and c.

A regression analysis showed that

- (1) there was no correlation between the total killed and the percentage necrophaged, ($r = -0.09$, not significant)
- (2) there was a slight negative correlation between the total killed and the percentage walled up ($r = -0.351$, $p, 0.05$), and
- (3) there was strong positive correlation between the total killed and the percentage unattended ($r = 0.881$, $p 0.001$).

Thus it was seen that the response to the cadavers followed the same pattern as in the previous section (7.4).

7.5a. Correlation between total dead and necrophagy as a result of arsenic poisoning

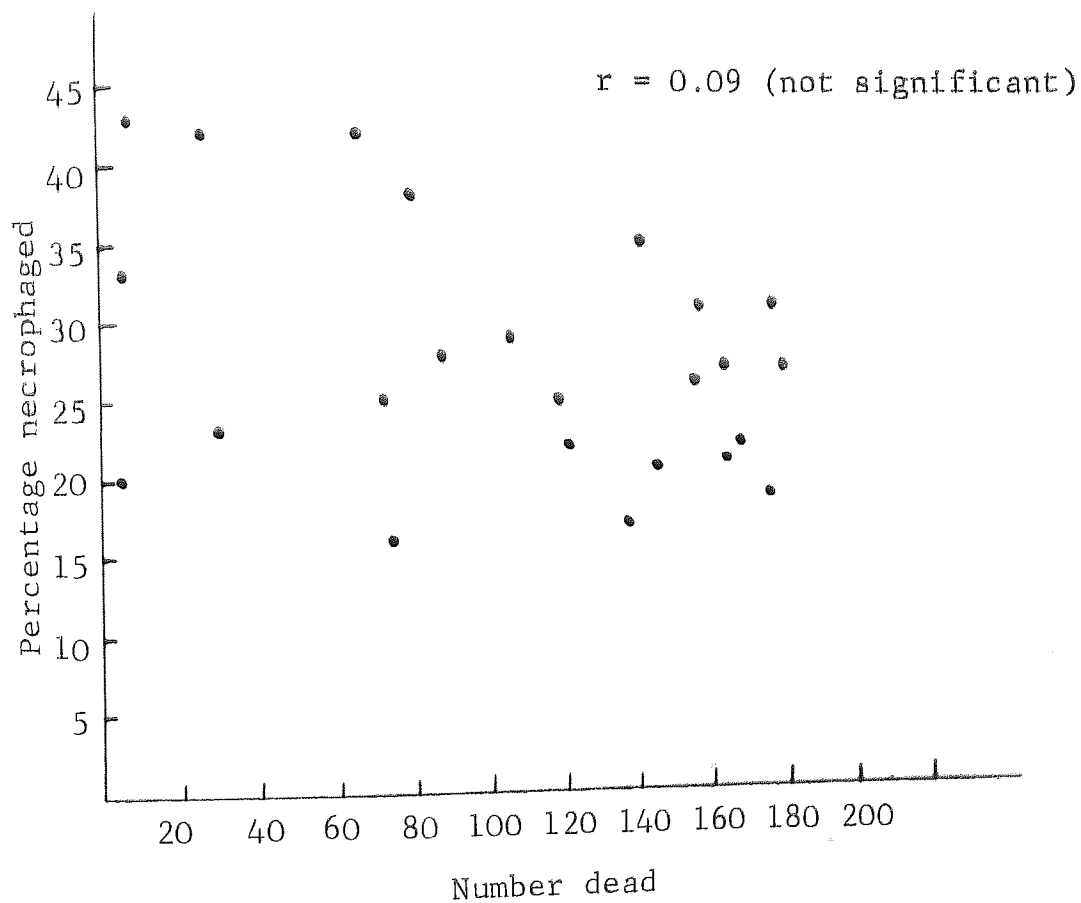
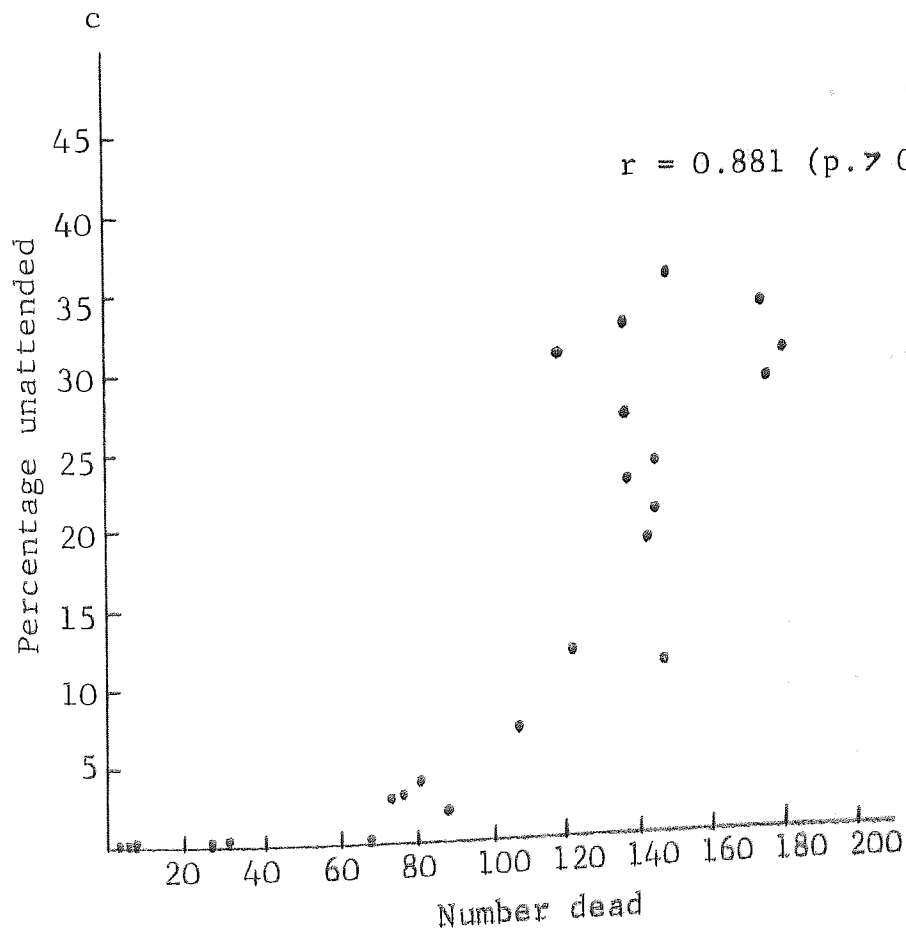
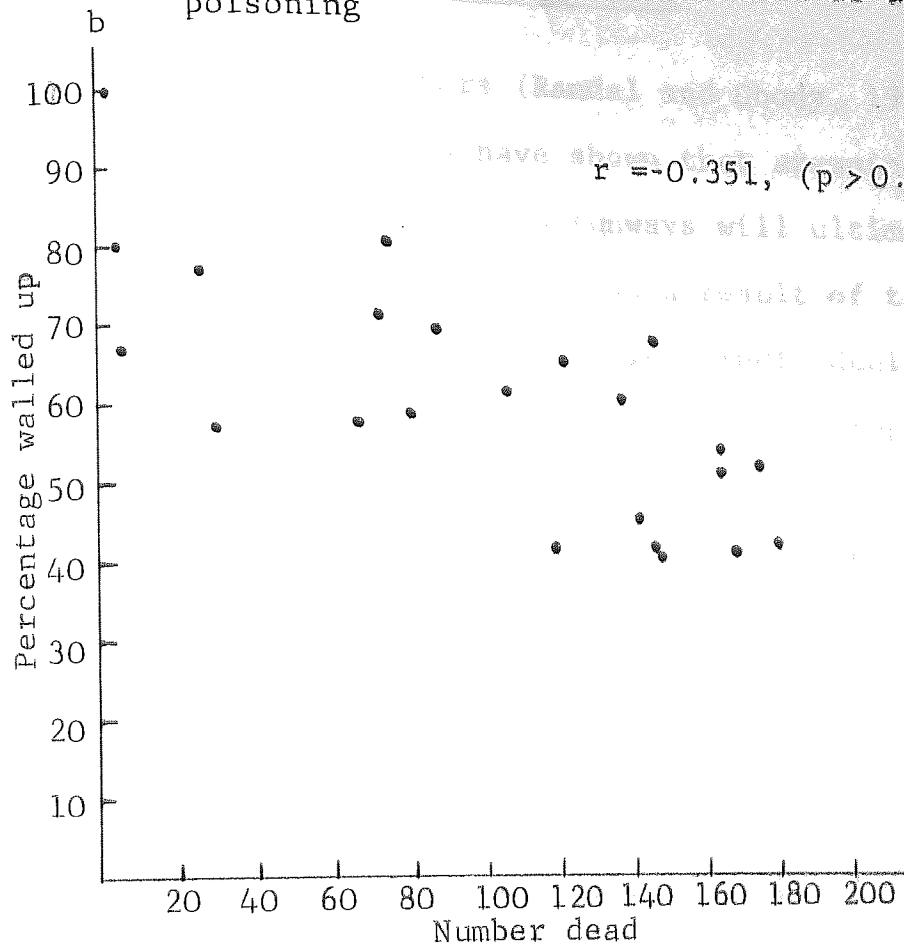


Fig. 7.5 (b & c) The correlation between total dead and (b) % walling up and (c) % unattended as a result of arsenic poisoning



7.5. Discussion and Conclusion

Several authors (Randal and Goody, 1934, Castle, 1934, Cahill, 1970) have shown that arsenical dusts placed in termite galleries and runways will ultimately be transmitted to the whole colony as a result of the mutual grooming and exchange of food between individuals of a colony. The poison was further spread by the necrophaging of dead nestmates as well. However, in the field, it has been found that, while dust treatments often brought temporary control, re-infestations were common suggesting that the dust treatments were unreliable and not permanent. This was often attributed to improper or inadequate use of poison. It was evident that the behavioural information on the means by which the poison was supposed to be circulated was also lacking.

Experiments, done during the course of this investigation on R. santonensis, and by other investigators on other species (Wilson, 1971) showed that olfactory stimuli, utilizing a limited array of chemical signals played a key role in bringing about interaction between individuals. This was also found to be the case in dealing with dying and dead nestmates.

Though a hypothesis was suggested that termites would be able to distinguish poisoned nestmates, subsequent experiments showed that, they did not appear to distinguish between nestmates carrying arsenic trioxide and those just isolated. They interacted normally with poisoned (living) individuals. Considering the fact that arsenic is both

tasteless and odourless it was not surprising that poisoned individuals were treated without distinction. This was also true in the case of arsenic-killed termites when they were fresh. However the latter were walled up within a very short time (sometimes as little as six hours) whereas those 'naturally dying' were not walled up until they began to decompose, typically after about ninety-six hours.

The problem here then is to account for the distinction shown between cadavers that were arsenic killed and those freeze killed. Foster (1949) reported that a number of species of Penicillium and Aspergillus were capable of breaking down arsenicals and releasing a volatile arsenic compound called "Gosio" gas. Since the termite environment and the termite body is rich in fungal spores (Sands, 1969), it is postulated here that the early walling up of arsenic-killed cadavers is due to the interaction of the fungi on the dead termite causing arsine to be given off thus making the cadaver repellant and possibly having some general toxic effect on the colony (i.e. fumigation). Much more work is obviously necessary to confirm this postulation.

From the results of the experiment conducted, the fate of a colony seemed to be dependant on the number of poisoned individuals returning to the nest. The greater the number returning, the greater the number of colony mates killed. Another important factor seemed to be the chemical nature of the poison. While odourless material did not interfere with the normal interaction between individuals of a colony those that possessed an odour or produced an odour through biotic and / or abiotic

interaction did. Thus nestmates carrying arsenic trioxide were able to transmit the poison as long as they were alive, but on death they rapidly ceased to do so because of the breakdown of arsenic trioxide. This difference between the living and dead arsenic-poisoned was quite clear from the experiments carried out. A Mann-Whitney U test was carried out between the total kill obtained through introducing dead nestmates (Table 7.2) and live nestmates carrying arsenic trioxide (Table 7.3). This showed that at all the five levels (0.5, 1, 2, 4 and 8%) the difference between the two was significant (p. 0.008, 0.075, 0.028, 0.028 and 0.028 respectively), the living being much more effective.

The total kill obtained during the experiment, described in this chapter, were very high. This was probably due to the fact that (1) the space available for the whole colony to effectively 'wall up' dying and dead nestmates was small and (2) the whole colony was contained in a fairly airtight container thus exposing them to the volatile breakdown products of arsenic trioxide. However in nature, subterranean termite nests with their diffused system of galleries and runways are spread over large areas. 'Protecting' parts of colonies from contamination either from poisonous vapours or poisoned nestmates is simply achieved by sealing up the runways and other interconnecting passages. This then can form the basis of a build up of populations causing re-infestation, especially in those species that have the capacity to produce replacement reproductives.

The above proposition has been made based on laboratory experiments. Obviously, much more work is needed under natural conditions to establish conclusively this hypothesis.

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CHAPTER VIIIGENERAL DISCUSSION

An attempt has been made in the present investigation to examine factors which influence trophallaxis, recognition of nestmates and cannibalism in the termite R. santonensis. The study of these factors was undertaken, in order to re-examine the basis on which control measures against subterranean termites in some parts of S.E. Asia and Australia are based. In those parts of the world finely ground toxic powders like arsenic trioxide are blown into galleries and runways of the termite, in the hope that foraging termites would pick the dust up and distribute it to the rest of the colony, through normal individual interaction on which the integration of social insect colonies depends. However, the all too frequent re-infestations (Howick, 1970) in areas subject to such control measures indicated that the information, on which the above control measures were based, might have been inadequate, and that the poisons were not reaching the whole colony.

Since it was considered that colony interaction and hence colony integration can only be understood by observing the behaviour of the individual, this investigation concentrated on understanding the individual behaviour of the subterranean termite R. santonensis. It must be emphasized that the entire study was carried out with laboratory colonies under laboratory conditions. Furthermore only one species of termite was used and it is not therefore justifiable to generalise from the results without further work on other species and

under field conditions. However, it is hoped that the investigation will contribute to a general understanding of some of the mechanisms involved in bringing about colony cohesion in termites..

To understand the behaviour of an organism it is essential to catalogue as complete a repertoire of behavioural acts as possible. Such a catalogue of behavioural acts for R. santonensis was described in Chapter 3. It was evident from the 'ethogram' that the 'workers' of this species performed all the routine functions (grooming others, food exchange, brood care, building, cannibalism, etc.) of the colony. This was further confirmed when it was established that workers formed the focal point in the distribution of food within the colony and hence in the distribution of any poisonous material.

Thus, using 'worker' individuals as experimental termites, an attempt was made to understand the factors or stimuli that released trophallactic behaviour. From experiments described in Chapter 4 (4.3) it was evident that internal (physiological) factors were important in determining the role, i.e. donor or acceptor, which an individual played. Termites that were hungry were found to solicit for food and those that were fed were found to donate food. However, in plate cultures, where there was plenty of food, individuals were normally found to alter between soliciting and donating even after one food exchange bout. It can only be postulated here that in a colony with plenty of food around it there must always be some food in the gut of all the termites, and shifting from acceptance to donation or

vice-versa, can be brought about, by one food exchange. Obviously this needs to be confirmed by more intensive work, on the sensory physiology of feeding, in termites. Though internal (or physiological) factors governed the need to donate or accept, they did not release trophallactic behaviour. It was the exteroceptive stimuli that released food exchange. Various experiments indicated that there was no specific 'food exchange releaser', such as head, antennal palpation, etc., in R. santonensis. Food donation was always directed towards the head, but unlike honeybees, where the presence of the antenna was always essential for food exchange (Free, 1956), termites were capable of exchanging food without the antennae. Antennal palpation seemed to act in an excitatory manner, and the antennae were used to orientate correctly for channelling food; and more important - with their chemoreceptors (Abushama, 1966) in 'identifying' potential participants in food exchange. It was found as a result of experiments described in Chapter 5, that 'identification' or 'recognition' of an individual was through odour and possession of a correct odour or combination of odours was essential in bringing about food exchange.

It was apparent from the work of earlier investigations (Andrews, 1911; Emerson, 1929; Verron, 1963), that like most social insects, termites are capable of recognizing individuals of alien colonies. Experiments described in Chapter 5 confirmed this in the case of R. santonensis. Although it had been postulated that the basis of recognition was colony odour, the experimental evidence for this was found to be lacking. The problem then was to account for

the factors that determined colony odour. If it is postulated that colony odour is determined by environmental factors like food then it is necessary to confirm whether colonies from the same nest when split into two and fed different foods would develop distinct odours. Chapter 5.3.4., described such an experiment which showed food is most probably the source of colony odour. However, in the experiment described, less than 60% were actually killed during the period of observation suggesting that food difference did not produce the total aggressive response normally exhibited towards an alien, (the fate of those that were not killed is not known clearly, but the fact that the termite cuticle is capable of 'adsorbing' odour (Emerson, 1929) might probably enable a good number of them to integrate successfully if they survive sufficiently long.). However, the fact that less than 60% of the individuals were subjected to aggression does not necessarily indicate that differences in food was not effective in causing differences in colony odour, but merely that diet alone was not responsible for the development of all the aspects of a distinct colony odour. It was then postulated that food or environmental factors played a modifying role in the establishment of colony odour but the decisive factor was possibly genetical. This was tested by experiments described in Chapter 5 and the results indicated that genetically different aliens on identical food provoked more aggression ($>40\%$ but $<50\%$ killed) than genetically similar aliens on different food ($>30\%$ $<40\%$ were killed) and genetically different aliens on different food provoked even more aggression ($>60\%$ killed) than the last two. Thus all the evidence in this investigation suggested very strongly that colony odour is multi-

factorial being basically inherited but considerably modified by environmental and metabolic factors. If indeed colony odour is inherited then it must be through the primary reproductives. If this were the case, an investigation into whether it is through the male or female might prove to be an interesting area for future research. It would also be interesting to find out whether the odour can be extracted or not.

Odours or olfactory stimuli were not only implicated in the interaction between living individuals, but also in the interaction between living individuals and dead nest-mates. Experiments described in Chapter 6 showed that chemical signals were responsible for signalling injury and death. Unlike most hymenopterans, which dispose their injured and dead by removing them to rubbish dumps (Wilson, 1971), termites eat them presumably in order to conserve the little nitrogen that is available to them (Moore, 1969). However, experiments and observations described in Chapter 6 showed that cadavers that did not respond to tactile stimuli and which gave out the 'correct' signal (odour) were disposed of by necrophaging and those that gave out 'strange' odours were disposed of by burial or 'walling up'. Experiments similar in design to those of Wilson, Durlach et al (1959), demonstrated that the necrophagic response was shown, to filter paper discs treated with low concentrations of fatty acids found in termite bodies (oleic, linoleic, palmitic) while higher concentrations of the same fatty acids either produced no detectable response or produced 'walling up' behaviour. All other compounds tested, viz. ammonium

sulphide, phenylethylamine, triethanolamine, etc., produced 'walling up' behaviour even in lower concentrations.

'Walling up' or burial behaviour showed all the behavioural elements involved in construction or building behaviour. Stuart (1967) showed that in termites, alarm, defence and construction were all interrelated and were a response to danger. For instance, the discovery of an intruder like a moving ant, which was considered as a high intensity stimulus evoked all three types of behaviour. The discovery of the alien was transmitted by 'alarm' behaviour which resulted in the recruitment of other members of the colony. This was followed by 'defense' when the moving ant was immobilized by killing it. The immobilized ant, was considered a low intensity stimulus and this evoked construction behaviour, viz. the deposition of faecal cement and debris around it till it was completely covered up thus neutralizing the important odour component of the stimulus. Hence the burying behaviour of unpalatable cadavers was very much a response to a low intensity 'danger' stimulus, and 'walling' them up was in effect an effort at removing a source of danger.

Since it was found, from the experiments already described, that the behavioural elements on which the dust treatment was based, were all brought about by chemical signals (or pheromones) between individuals; it was considered that nestmates carrying or killed by toxicants would be treated as alarm-causing objects and recognised, killed and buried. This would then prevent the spread of the

toxicants to the rest of the colony. However, experiments, described in Chapter 7, showed that nestmates carrying poison (AS_2O_3) were initially accepted and treated like any other nestmate. Similarly nestmates killed by AS_2O_3 and still fresh were also treated as any other corpse and thus necrophaged. Such an initial acceptance of poisoned nestmates caused high mortalities (dependant on the number of termites that were initially poisoned) in the colonies. But with increasing deaths it was found that the colony was incapable of eating all the dead while they were still fresh; and arsenic-killed cadavers became repellant within 6 hours (in some cases) and these were walled up. An examination of all the colonies subjected to experimentation revealed that up to 52 % were walled up and only 28% were actually eaten.

The problem then is to explain the lack of repellency in the first few hours after death of arsenic poisoned cadavers. It can only be postulated here that whereas arsenic lacks any apparent taste or odour in its natural state, it can be broken down to release odoriferous compounds like Arsine or "Gosio gas" by certain fungi (Foster, 1949). Although this seems probable in the present case, obviously it needs to be investigated. Esenther (personal communication) observed that Mirex-killed (an organo phosphorous insecticide) Reticulitermes were 'walled up' as well by their nestmates in laboratory studies.

The release of a poisonous compound can also explain the high rate of mortalities that occurred in the plate nests of the present investigation - where the colonies

were kept in fairly air tight containers. Thus, whereas initial deaths in colonies were due to actual contact those that occurred later must be presumed to be due to fumigation.

If arsenic trioxide treatment does develop in this manner then the fact that reinfestations are a common phenomenon in the field could be explained as follows:- On the application of the poisonous dust there is a cessation of activity due to the initial action of the poison. However, with the onset of defensive behaviour, i.e. walling off of cadavers and areas having an offensive odour, by the surviving members of the colony a substantial percentage of the population of a colony can survive (experiments conducted during the course of this investigation bore this out; and it is worthwhile to note that very often the survivors included the replacement reproductives), to rebuild the colony again, thus restoring its capacity to initiate further infestations. More work is obviously necessary, especially under field conditions, to establish this conclusively. Such work should not only be confined to dust treatments but also to other toxicants used against termites.

... on the antennal olfac-

... *terrapenis argus*...

REFERENCES

REFERENCES

Abushama, F.T. (1966)

Electrophysiological investigations on the antennal olfactory receptors of the dampwood termite Zootermopsis angusticollis.

Entomologia Experimentalis et Applicata, 9 (3): 343-348.

Adam, Brother (1951)

Introduction of queens.

Proceedings of the 14th International Bee Keeping Congress, 10: 1-5.

Adamson, A.M. (1941)

Laboratory techniques for the study of living termites.

Ecology, 22: 411-414.

Alibert, J. (1959)

Les échanges trophallactiques chez le termite à cou jaune ("Calotermes flavicollis" Fabr.) étudiés à l'aide du phosphore radioactif.

Compte Rendu de l'Académie des Sciences, Paris, 248: 1040-1042.

Alibert, J. (1960)

Les échanges trophallactiques entre Termites sexués et larves de jeunes fondations de colonies et de sociétés plus âgées (Calotermes flavicollis Fabr.)

Compte Rendu de l'Académie des Sciences Paris, 250: 4205-4207.

Alibert, J. (1963)

Echanges trophallactiques chez un termite superieur.

Contamination par le phosphore radio-actif de la population d'un nid de Cubitermes fungifaber.

Insectes Sociaux, 10: 1-12.

Alibert, J. (1965)

Mue et trophallaxie proctodéale chez Calotermes flavicollis.

Compte Rendu de l'Academie des Sciences Paris, 261: 3207-3210.

Alibert, J. (1968)

Influence de la société et de l'individu sur la trophallaxie chez Calotermes flavicollis Fabr. et Cubitermes fungifaber (Isoptera).

Colloques Internationaux du Centre National de la Recherche Scientifique, Paris, 1967, No. 173: 237-288.

Alibert, J.B. (1969)

Trophallaxy of the yellow-necked termite, Kalotermes flavicollis Fabr. studied using radio-isotopes. I. Participation of the different castes of the overall trophallaxy. The categories of food transmitted.

Annales des Sciences Naturelles, 2: 234-325 (In French).

Allen, M.D. (1955)

Observations on honey bees attending their queen.

British Journal of Animal Behaviour, 3 (2): 66-69.

Allen, M.D. (1956)

The behaviour of honey bees preparing to swarm.

British Journal of Animal Behaviour, 4 (1): 14-22.

Andrew, B.J. (1930)

Method and rate of protozoan refaunation in the termite
Termopsis angusticollis Hagen.

University of California (Berkeley) Publications in Zoology,
33: 449-470.

Andrews, E.A. (1911)

Observations on termites in Jamaica.

Journal of Animal Behaviour, 1 (3): 193-228.

*Beaumont, J. (1889-1890)

Observations on the termites or white ants of the isthmus of
Panama.

Transactions of the New York Academy of Science, 8: 85-114;
9: 157-180.

Becker, G. (1961)

Beiträge zur Prüfung und Beurteilung der natürlichen
Dauerhaftigkeit von Holz gegen Termiten.

Holz Roh-Werkstoff, 19: 278-290.

Becker, G. (1965a)

Feuchtigkeitseinfluss auf Nahrungswahl und-verbrauch einiger
Termiten-Arten.

Insectes Sociaux 12: 151-181.

Becker, G. (1965b)

Versuche über den Einfluss von Braunfäulepilzen auf Wahl und Ausnutzung der Holznahrung durch Termiten.

Materials und Organismen, 1: 95-156.

Becker, G. (1969)

Rearing and Testing Methods in the laboratory.

In. K. Krishna and F.M. Weesner eds. (q.v.) Biology of Termites Vol. 1. pp. 351-385.

Becker, G. (1971)

Reticulitermes (Ins. Isopt.) in Central and Western Europe.

Mintech. Translation T6617, 13pp.

*Bethe, A., (1898)

Dürfen wir den Ameisen und Bienen psychische Qualitäten zuschreiben?

Pflügers Archiv für die Gesamte Physiologie, 70: 15-100.

Blum, M.S. (1970)

The chemical basis of insect sociality.

In. M. Berzosa, ed. Chemicals Controlling insect behaviour, pp. 61-94.

Brun, R. (1912)

Zur psychologie der Künstlichen Allianzkolonien bei den Ameisen.

Biologisches Zentralblatt, 32: 305-322.

Bouillon, A. (1970)

Termites of the Ethiopian region.

In. K. Krishna and F.M. Weesner eds. (q.v.) Biology of termites Vol. II, pp. 153-280.

Buchli, H.H.R. (1950)

Recherche sur la fondation et le développement des nouvelles colonies chez le termite lucifuge (Reticulitermes lucifugus Rossi).

Physiologia Comparata et Oecologia, 2: 145-160.

Buchli, H.H.R. (1958)

L'origine des castes et les potentialités ontogéniques des termites européens du genre Reticulitermes.

Annales des Sciences Naturelles, (11) 20 (18): 263-429.

Buchli, H.H.R. (1961)

Les relations entre la colonie maternelle et les jeunes imagos ailes de Reticulitermes lucifugus.

Vie Milieu, 12: 627-632.

Butler, C.G. (1954)

The method and importance of the recognition by a colony of honey bees (A. mellifera) of the presence of its queen.

Transactions of the Royal Entomological Society, London 105:11-29.

Butler, C.G. (1963)

Pheromones in sexual processes in insects.

Symposium of the Royal Entomological Society, London, 2: 66-77.

Butler, C.G. and Free, J.B. (1952)

The behaviour of worker honeybees at the hive entrance.

Behaviour 4 (4): 262-292.

Buttel-Reepen, H. Von. (1900)

Sind die Bienen "Reflexmaschinen"? Experimentelle Beiträge zur Biologie der Honigbiene.

Biologisches Zentralblatt, 20: 97-109.

Cahill, T.D. (1970)

Permanence and durability of treatments

Proceedings of the Termite Conference and Instruction Course for the Victorian pest control industry, Australia, pp 59-66.

Carter, F.L. and Dinus, L.A. and Smythe, R.V. (1972)

Fatty acids of the Eastern subterranean termite Reticulitermes flavipes (Isoptera: Reticulitermitidae).

Annals of the Entomological Society of America, 65 (3): 655-658.

Castle, G.B. (1934)

The damp-wood termites of western United States genus

Zootermopsis (formerly Termopsis).

In C.A. Kofoed et al., ed., Termites and termite control, 2nd ed. University of California Press, Berkeley, pp. 273-310.

Chauvin, R. (1968)

Animal societies from the bee to the gorilla.

(English tr. by G. Ordish). Hill and Wang, New York. 281pp.

Cook, S.F. and Scott, K.G. (1933)

The nutritional requirements of Zootermopsis angusticollis.
Journal of Cellular and Comparative Physiology, 4: 95-110.

Deither, V.G. and Stellar, E. (1961)

Animal Behaviour.

Prentice-Hall, Inc., New Jersey, 118pp.

Donisthorpe, H. St. J.K. (1927)

The guests of British ants, their habits and life histories.

George Routledge & Sons Ltd., London. 244pp.

Emerson, A.E. (1929)

Communication among termites.

Transactions of the 4th International Congress of Entomology
1928 Ithaca, 2: 722-727.

Emerson, A.E. (1939)

Social coordination and the superorganism.

American Midland Naturalist, 21: 182-209.

*Escherich, K. (1909)

"Die Termiten" Klinkhardt, Leipzig.

Esenther, G.R., Allen, T.C., Casida, J.E. Schenefelt, R.D.
(1961)

Termite attractant from fungus-infected wood.

Science 134: 50.

Feytaud, J. (1912)

Contribution à l'étude du Termite lucifuge.

Archives d'Anatomie Microscopique et de Morphologie Expérimentale, 13: 481-607.

Feytaud, J. (1925)

Existe-t-il plusieurs races de Reticulitermes lucifugus Rossi?

Revue de Zoologie Agricole et appliquée, 24: 161-164.

Feytaud, J. (1948)

A propos des Reticulitermes de France.

Proceedings Eighth International Contress of Entomology,
Stockholm, pp. 380-381.

Field, A.M. (1904)

Power of recognition among ants.

Biological Bulletin, Marine Biological Laboratory, Woods
Hole, 7 (5): 227-250.

Finney, D.J. (1971)

Probit analysis.

Cambridge University Press. 333pp.

*Forel, A. (1874)

Les fourmis de la Suisse.

Société Helvétique des Sciences Naturelles, Zurich. 452pp.

Foster, J.W. (1949)

Chemical activities of fungi.

Academic Press, New York. 648pp.

Free, J.B. (1955)

The behaviour of egg laying workers of bumblebee colonies.
British Journal of Animal Behaviour 3 (4): 147-153.

Free, J.B. (1956)

A study of the stimuli which release the food begging and offering responses of worker honeybees.
British Journal of Animal Behaviour, 4 (3): 94-101.

Free, J.B. (1957)

The transmission of food between worker honeybees.
British Journal of Animal Behaviour, 5 (2): 41-47.

Free, J.B. (1958)

The defense of bumblebee colonies.
Behaviour 12 (3): 233-242.

Free, J.B. (1959)

The transfer of food between the adult members of a honey-bee community.
Bee World, 40 (8): 193-201.

Frisch, K. von and Rösch, G.A. (1926)

Neue Versuche über die Bedeutung von Duftorgan und Pollenduft für die verständigung im Bienenvolk.
Zeitschrift für Vergleichende Physiologie, 4(1): 1-21.

Fuller, C. (1915)

Observations on some South African termites.
Annals-Natal Museum, 3: 329-505.

Gay, F.J. and Calaby, J.H. (1970)

Termites of the Australian region.

In. K. Krishna and F.M. Weesner eds. (q.v.) Biology of termites Vol. II, pp. 393-448.

Gay, F.J., Greaves, T, Holdaway, F.G. and Wetherly, A.H. (1955)
Standard laboratory colonies of termites for evaluating the resistance of timber, timber preservatives and other materials to termite attack.

Australia, Commonwealth Scientific and Industrial Research Organization Bulletin, 277: pp. 67.

*Goetsch, W. (1936)

Beiträge zur biologie des termitenstaates.

Zeitschrift für Morphologie und ökologie der Tiere, 31:
490-560.

Gossword, K. (1962)

On the methods of testing materials for termite resistance with particular consideration of the physiological and biological data of the test technique.

In. Proceedings of the New Delhi Symposium, 1960, Termites in the humid tropics. UNESCO, Paris. pp. 169-178.

Gossword, K. and Kloft, W. (1958)

Radioaktiv Isotope zur Erforschung des staatenlebens der insekten.

Über die Fortschritte in Wissenschaft und Technik, 58: 743-745.

Grassé, P.P. (1939)

La construction du nid et le travail collectif chez les Termites supérieurs.

Journal de Psychologie normale et pathologique, (Juillet-December): 370-396.

Grassé, P-P. (1942)

L'essaimage des Termites: Essai d'analyse causale d'un complex instinctif.

Bulletin Biologique de la France et de la Belgique, 76 (4): 347-382.

Grasse, P.P. (1949)

Ordre des Isoptères ou Termites.

In. P.-P. Grassé, ed., Traité de Zoologie, Vol. 1X Mason et Cie, Paris. pp. 408-544.

Grasse, P.-P. (1952)

L'effet de groupe chez les insectes.

Bulletin de la Section France, Union International pour l'etude des Insectes Sociaux, 1: 32-43.

Grassé, P.-P. (1959)

La reconstruction du nid et les co-ordination inter-individuelles chez Bellicositermes natalensis et Cubitermes sp.

La théorie de la stigmergie: Essai d'interpretation du comportement des Termites constructeurs.

Insect Sociaux, 6: 41-84.

Grassé, P.-P. and Noirot, C. (1945)

La transmission des flagellés symbiotiques et les aliments des termites.

Bulletin Biologique de la France et de la Belgique, 79 (4): 273-292.

Grassi, B. and Sandias, A. (1896-1897)

The constitution and development of the society of termites: Observations on their habits; with appendices on the parasitic protozoa of Termitidae, and on the Embiidae.

Quarterly Journal of Microscopical Science, 39 (155): 245-322 (English translation).

Greaves, T. (1962)

Studies of foraging galleries and the invasion of living trees by Coptotermes acinaciformis and C. brunneus (Isoptera).

Australian Journal of Zoology, 12(2): 250-262.

Greaves, T., McInnes, R.S. and Dowse, J.E. (1967)

The control of termites (Coptotermes spp.) in Blackbutt (Eucalyptus pilularis).

Commonwealth Scientific and Industrial Research Organization, Australia. Division of Entomology, Technical Paper 7: 35-43.

Harris, W.V. (1961)

Termites, their recognition and control.

Longmans, Green & Co. Ltd., London. 187pp.

Harris, W.V. (1965)

Recent developments in termite control.

Pest Articles News Summaries: (A) 11: 33-43.

Harris, W.V. and Sands, W.A. (1965)

The social organization of termite colonies.

Symposium of the Zoological Society of London, 14: 113-131.

Haskins, C.P. and Haskins, E.A. (1950)

Notes on the biology and social behaviour of the archaic ponerine ants of the genera Myrmecia and Promyrmecia.

Annals of the Entomological Society of America, 43 (4): 461-491.

Heath, H. (1927)

Caste formation in the termite genus Termopsis.

Journal of Morphology and Physiology, 43: 387-425.

Hendee, E.C. (1935)

The role of fungi in the diet of the common dampwood termite

Zootermopsis angusticollis.

Hilgardia, 9: 499-525.

Heyde, K. (1924)

Die Entwicklung der psychischen Fähigkeiten bei Ameisen und ihr Verhalten bei abgeänderten biologischen Bedingungen.

Biologisches Zentralblatt, 44 (11): 623-654.

Hickin, N. (1971)

Termites: A world problem.

Hutchinson, The Rentokil Library, London. 232pp.

Hill, G.F. (1925)

Notes on Mastotermes darwiniensis Froggatt (Isoptera).
Proceedings of the Royal Society, Victoria (N.S.), 37:
 119-124.

Hinde, R.A. (1966)

Animal Behaviour, a synthesis of ethology and comparative psychology.

McGraw-Hill Book Company, New York. 534pp.

+

*Hoffman, I. (1961)

Über die Arbeitsteilung in weiselrichtigen und weisellosen
 Kleinvölkern der Honigbiene.

Zeitschrift für Bienenforschung, 5: 267-278.

*Holmgren, N. (1912)

Termitenstudien. 4 Versuch einer systematischen Monographie
 der Termiten der orientalischen Region.

Kungliga Svenska Vetenskapsakademiens Handlingar, 48 (4):
 136-151.

Howse, P.E. (1964a)

The significance of the sound produced by the termite

Zootermopsis angusticollis Hagen.

Animal Behaviour, 12: 284-300.

Howse, P.E. (1964b)

An investigation into the mode of action of the subgenual
 organ in the termite Zootermopsis angusticollis Emerson, and

in the cockroach, Periplaneta americana L.

Journal of Insect Physiology, 10: 409-424.

Howse, P.E. (1965)

On the significance of certain oscillatory movements of termites.

Insectes Sociaux, 12: 335-346.

Howse, P.E. (1970)

Termites: A study in social behaviour.

Hutchinson University Library, London. 150pp.

Hrdy, I. (1964)

Laboratory methods for testing the resistance of materials against termites. (Russian; English summary).

Rozpravy Ceskoslovenske akademie ved, 74: 1-147.

Humphries, E.C. (1956)

Mineral components and ash analysis.

In. Paech, K. and F.M. Tracey eds. Modern methods of plant analysis vol. I. pp. 468-502.

Hungate, R.E. (1941)

Experiments on the nitrogen economy of termites.

Annals of the Entomological Society of America, 34: 467-489.

Imms, A.D. (1919)

On the structure and biology of Archotermopsis, together with descriptions of new species of intestinal Protozoa and general observations on the Isoptera.

Philosophical Transaction of the Royal Society, (B) 209: 75-180.

Kalamus, H. and Ribbands, G.R. (1952)

The origin of odours by which honeybees distinguish their companions.

Proceedings of the Royal Society, (B) 140: 50-59.

Kalshoven, R.G.E. (1930)

Bionomics of Kalotermes tectonae Damm. as a base for its control (in Dutch).

H. Veenman and Zonen, Wageningen. 154pp.

Karlson, P. and Butenandt, A. (1959)

Pheromones (ectohormones) in insects.

Annual Review of Entomology, 4: 39-58.

Kistner, D.H. (1969)

The biology of termitophiles.

In. K. Krishna and F.M. Weesner eds. (q.v.) Biology of termites Vol. I, pp. 525-557.

Köhler, F. (1955)

Wache und Volksduft im Bienestaat.

Zeitschrift für Bienenforschung, 3: 57-63.

Lange, R. (1960)

Über die Futterweitergabe Zwischen Angehörigen verschiedener Waldameisenstaaten.

Zeitschrift für Tierpsychologie, 17 (4): 389-401.

Lange, R. (1967)

Die Nahrungsverteilung unter den Arbeiterinnen des Waldameisenstaates.

Zeitschrift für Tierpsychologie, 24 (5): 513-545.

Lash, J.W. (1952)

A new species of Reticulitermes (Isoptera) from Jerusalem, Palestine.

American Museum Novitates, New York. No. 1575: 1-7.

Lecomte, J. (1952)

Hétérogénéité dans le comportement agressif des ouvrières d'Apis mellifica.

Compte Rendu de l'Academie des Sciences, Paris, 234 (8): 890-891.

*Lespès, C. (1856)

Recherches sur l'organisation et les moeurs du Termite lucifuge.

Annales des sciences naturelles. Paris, 5 (4): 227-282.

++

Light, S.F. (1944)

Experimental studies on ectohormonal control of the development of supplementary reproductives in the termite genus Zootermopsis (formerly Termopsis).

University of California Publications in Zoology, 43 (17): 413-454.

Lubbock, J. (1894)

Ants, bees and wasps: A record of observations on the habits of the social Hymenoptera.

D. Appleton & Co., New York. 448pp.

Lund, A.E. (1967)

The study of subterranean termites.

Record of the British Wood Preserving Association, 119-127

Lüscher, M. (1949)

Continuous observation of termites in laboratory cultures.
Acta Tropica, 6: 161-165.

Lüscher, M. (1952)

Die Produktion und Elimination von Ersatz geschlechtstieren
bei der Termite Kaloterme flavicollis Fabr.
Zeitschrift für Vergleichende Physiologie, 34 (2): 123-141.

Lüscher, M. (1955)

Zur Frage der Übertragung sozialer Wirkstoffe bei Termiten.
Naturwissenschaften, 42: 86.

Lüscher, M. (1958)

Experimentelle Erzeugung von Soldaten bei der Termite
Kaloterme flavicollis.
Naturwissenschaften, 45: 69-70.

Lüscher, M. (1960)

Hormonal control of caste differentiation in termites.
Annals of the New York Academy of Sciences, 89: 549-563.

Lüscher, M. (1961)

Social control of polymorphism in termites.
Symposium of the Royal Entomological Society of London, 1:
57-67.

Lüscher, M. (1962)

Hormonal regulation of development in termites.
Symposia Genetica et Biologica Italica, 10: 1-11.

Lüscher, M. (1964)

Die spezifische Wirkung männlicher und weiblicher Ersatzgeschlechtstiere auf die Entstehung von Ersatzgeschlechtstieren bei der Termiten Kaloterme flavicollis (Fabr.).
Insectes Sociaux, 11: 79-90.

Mathieu, H. (1959)

The unrecognized scourge: The termite (in French).
J.B. Baillere and Son, Paris. 93pp.

Mathieu, H. (1962)

Die termiten.

Holzforschung und Holzverwertung, 14: 97-103.

McCook, H.C. (1879)

Combats and nidification of the pavement ant Tetramorium caespitum.

Proceedings of the Academy of Natural Sciences of Philadelphia, 1879, 31: 151-161.

McMahan, E.A. (1963)

A study of termite feeding relationships, using radioisotopes.

Annals of the Entomological Society of America, 56: 74-82

McMahan, E.A. (1966a)

Studies of termite wood-feeding preferences.

Proceedings of the Hawaiian Entomological Society. 19(2):
237-48.

McMahan, E.A. (1966b)

Food transmission within the Cryptotermes brevis colony
(Isoptera: Kalotermitidae). *Annales du genre*, 1966, 1: 1-10.

Annals of the Entomological Society of America, 59 (6):
1131-1137.

McMahan, E. (1969)

Feeding relationships and radio isotope techniques.

In. K. Krishna and F.M. Weesner eds. (q.v.). Biology of
termites, Vol. 1. pp. 387-406.

Miller, E.M. (1942)

The problem of castes and caste differentiation in Prorhin-
otermes simplex.

Bulletin of the University of Miami 15: 1-27.

Miller, M. (1969)

Caste differentiation in the lower termites.

In. K. Krishna and F.M. Weesner, eds. (q.v.). Biology of
Termites, Vol. 1. pp. 283-310.

Moore, B.P. (1969)

Biochemical studies in termites.

In. K. Krishna and F.M. Weesner, eds. (q.v.). Biology of
termites. Vol. 1. pp. 407-432.

Montagner, H. (1963a)

Contribution à l'étude de déterminisme des castes chez les
Vespides.

Compte Rendu de la Société Biologie, Paris, 157(1): 147-150.

Montagner, H. (1963b)

Étude préliminaire des relations entre les adultes et le couvain chez les guêpes sociales du genre Vespa, au moyen d'un radio-isotope.

Insect Sociaux, 10(2): 153-165.

Montagner, H. and Courtouis, G. (1963)

Données nouvelles sur le compartiment alimentaire et les échanges trophallactiques chez les guêpes sociales.

Compte Rendu de l'Académie des Sciences, Paris, 256 (19): 4092-4094.

*Montagner, H. (1966)

Le mécanisme et les conséquences des comportements trophallactiques chez les guêpes du genre Vespa.

Thèses, Faculté des Sciences de l'Université de Nancy, France, 143 pp. (quoted by Wilson, 1971).

Morgan, F.D. (1959)

The ecology and external morphology of Stolotermes ruficeps Brauer (Isoptera, Hodotermitidae).

Transactions of the Royal Society New Zealand, 86: 155-195.

*Mueller, F. (1873)

Beiträge zur Kenntnis der Termiten. IV. Die Larven von Calotermes rugosus Hagen.

Zeitschrift für Naturwissenschaften, Jena, 7: 333-358.

Nel, J.J.C. (1968)

Aggressive behaviour of the harvester termites Hodotermes mossambicus (Hagen) and Trinervitermes trinervoides (Sjöstedt).

Insectes Sociaux, 15(2): 145-156.

Nel, J.J.C., Hewitt, P.H., and Smith, J.L. (1969)

The behaviour of the Harvester Termite (Hodotermes mossambicus (Hagen)) in a laboratory colony.

Journal of the Entomological Society of South Africa, 32: 9-24.

Noirot, C. (1952)

Les soins et l'alimentation des jeunes chez les termites.
Annales des Sciences Naturelles, (11) 14 (2-4): 405-414.

Noirot, C. (1955)

Recherches sur le polymorphisme des termites supérieurs
(Termitidae)

Annales des Sciences Naturelles, (11) 17 (3-4): 399-595.

Noirot, C. (1969)

Glands and Secretions.

In. K. Krishna and F.M. Weesner eds. (q.v.). Biology of Termites vol. 1. pp. 89-123.

Noirot, C. and Noirot-Timotheé, C. (1969)

The digestive system.

In. K. Krishna and F.M. Weesner eds. (q.v.) Biology of Termites vol. 1. pp. 387-406.

Nutting, W.L. (1969)

Flight and colony foundation.

In. K. Krishna and F.M. Weesner, eds. (q.v.), Biology of Termites Vol. 1. pp. 232-288.

Pickens, A.L. (1932)

Observations on the genus Reticulitermes Holmgren.

Pan-Pacific Entomologist, 8: 178-180.

Pickens, A.L. (1934)

The biology and economic significance of the western subterranean termite Reticulitermes hesperus.

In. C.A. Kofoed et al (ed). Termite and Termite Control.

2nd ed. University of California Press, California.

pp. 184-186.

Randall, M. and Goody, T.C. (1934)

Poisonous dusts: 1. Treatments with poisonous dusts.

In. Kofoed, C.A. et al ed. Termites and termite control.

(2nd ed). University of California Press. pp. 433-449.

Ratcliffe, F.N., Gay, F.J. and Greaves, T. (1952)

Australian termites. The biology, recognition and economic importance of the common species.

Commonwealth Scientific and Industrial Research Organization.

Melbourne. 124pp.

Rau, P. (1930)

Animosity and tolerance in several species of Polistes wasps.

Journal of Comparative Psychology, 10 (3): 267-286.

Richard, G. (1950)

Le phototropisme du termite á cou jaune (Calotermes flavi-
collis Fabr.) et ses bases sensorielles.

Annales des Sciences Naturelles, (11) 12 (3-4): 485-603.

Richard, G. (1969)

Nervous system and sense organs.

In. K. Krishna and F.M. Weesner, eds. (q.v.), Biology of
termites, Vol. 1. pp. 161-192.

Ribband, C.R. (1952)

Division of labour in the honeybee community.

Proceedings of the Royal Society, (B) 140: 32-43.

Ribbands, C.R. (1953)

The behaviour and social life of honeybees.

London Bee Research Association.

Ribbands, C.R. (1955)

The scent perception of the honeybee.

Proceedings of the Royal Society, (B) 143: 367-379.

Ribbands, C.R. (1965)

The role of recognition of comrades in the defense of
social insect communities.

Symposium of the Zoological Society of London, 14: 159-168.

Ribbands, C.R., Kalamus, H. and Nixon, H.L. (1952)

New evidence of communication in the honeybee colony.

Nature, London, 170: 438-440.

Rupli, E. (1969)

Die elimination uberzahliger ersatzgeschlechtstiere beider
termiten Kaloterme flavicollis (Fabr.)

Insectes Sociaux, 16 (3): 235-248.

Sakagami, S.F. (1954)

Occurrence of an aggressive behaviour in queenless hives,
with considerations on the social organization of honeybee.

Insectes Sociaux, 1: 331-343.

Sands, W.A. (1969)

The association of termites and fungi.

In. K. Krishna and F.M. Weesner eds. (q.v.) Biology of
termites Vol. 1. pp. 495-524.

Sands, W.A. (1973)

Termites as pests of tropical food crops.

Pest Articles and News Summaries, 19 (2): 167-177.

Schneirla, T.C. (1958)

The behaviour and biology of certain neartic army ants,
last part of the functional season.

Insectes Sociaux, 5 (2): 215-255

Siegel, S. (1956)

Non Parametric Statistics for the Behavioural Sciences.

McGraw-Hill Book Company, New York. 312pp.

Skaife, S.H. (1955)

Dwellers in Darkness. Location of Alphas
Longmans and Green Co. Ltd. New York 134pp.

*Smeathman, H. (1781)

Some accounts of the termites which are found in Africa
and other hot climates.

Philosophical Transactions of the Royal Society, (B) 71:
139-142.

Smythe, R.V. and Coppel, H.C. (1966)

Some termites may secrete trail-blazing attractants to
lead others to food sources.

Pest Control, 34 (10): 73-78.

Spear, P.J. (1970)

Principles of termite control.

In. K. Krishna and F.M. Weesner eds. (q.v.) Biology of
termites Vol. II: 577-604.

*Stuart, A.M. (1960)

Experimental studies on communication in termites

Ph.D. Thesis, Harvard University (quoted by Stuart, A.M.,
1969).

Stuart, A.M. (1961)

Mechanism of trail laying in two species of termites.

Nature 189: 419.

Stuart, A.M. (1963)

Studies on the communication of alarm in the termite Zootermopsis nevadensis (Hagen), Isoptera.

Physiological Zoology, 36: 85-96.

Stuart, A.M. (1964)

The structure and function of the sternal gland in Zootermopsis nevadensis (Hagen) Isoptera.

Proceedings of the Zoological Society of London, 143 (1): 43-52.

Stuart, A.M. (1967)

Alarm, defence and construction behaviour relationships in termites (Isoptera).

Science, 156: 1123-1125.

Stuart, A.M. (1969)

Social Behaviour and Communication.

In. K. Krishna and F.M. Weesner, eds. (q.v.), Biology of termites, Vol. I. pp. 193-232.

* Taranov, G.F. and Ivanov, L.V. (1946)

Observations on the behaviour of the queen in the colony
(Russian)

Pchelovodstro 23 (2/3): 35-39. (quoted by Free, J.B., 1959).

Thurlich, L. (1960)

Vergleichende morphologische Betrachtung der Soldaten der europäischen und vorderasiatischen Reticulitermes-Arten. Entomologische Mitteilungen aus dem Zoologischen Staatsinstitut u. Zoologischen Museum, Hamburg, 30: 145-161.

Verron, H. (1963)

Rôle des stimuli chimiques dans l'attraction sociale chez Calotermes flavicollis (Fabr.). Insectes Sociaux, 10(2): 167-184, 3: 185-296.

Vishnoi, H.S. (1956)

The structure, musculature and mechanisms of the feeding apparatus of the various castes of the termite Odontotermes obesus (Rambur). Part 1. Clypeo-labrum. Journal of the Zoological Society of India, 8: 1-18.

Vowles, D.M. (1952)

Individual behaviour patterns in ants. Advancement of Science, 10: 18-21.

Vowles, D.M. (1955)

The foraging of ants. British Journal of Animal Behaviour, 3: 1-13.

UNESCO (1962)

Termites in the humid tropics. Proceedings of the New Delhi Symposium. 1960 UNESCO, Paris. 259pp.

Wallis, D.I. (1961)

Food sharing behaviour of the ants Formica sanguinea and Formica fusca.

Behaviour, 17 (1): 17-47.

Wallis, D.I. (1962)

Behavioural patterns in the ant, Formica fusca.

Animal Behaviour, 10: 105-111.

Wallis, D.I. (1962)

Aggressive Behaviour in the ant, Formica fusca.

Animal Behaviour, 10 (3-4): 391-408.

Wallis, D.I. (1964)

Aggression in social insects.

Symposium of the Institute of Biology, London, 13: 15-22.

Weesner, F.M. (1960)

Evolution and biology of the termites.

Annual Review of Entomology, 5: 153-170.

*Weyer, F. (1930)

Über Ersatzgeschlechtstiere bei Termiten.

Zeitschrift für Morphologie und Ökologie der Tiere, 19:

364-380.

Wheeler, W.M. (1910)

Ants: Their structure, development and behaviour.

Columbia University Press, New York, pp. 663.

Wheeler, W.M. (1918)

A study of some ant larvae with a consideration of the origin and meaning of social habits among insects.

Proceedings of the American Philosophical Society, 58 (1): 1-40.

Wheeler, W.M. (1928)

The social insects: Their origins and evolution.

Kegain, Paul, Trench, Trubner & Co. Ltd., London. 378pp.

Williams, C.M. (1952)

Physiology of Insect diapause. IV. The brain and prothoracic glands as an endocrine system in the cecropia silkworm.

Biological Bulletin, 103: 120-138.

Williams, R.M.C. (1959)

Flight and colony foundation in two *Cubitermes* species

(Isoptera: Termitidae).

Insectes Sociaux, 6 (2) 203-218.

Wilson, E.O. (1962)

Chemical communication among workers of the fire ant

Solenopsis saevissima (Fr. Smith). 1. The organization of mass foraging.

Animal Behaviour, 10: 134-147.

Wilson, E.O. (1965)

Chemical communication in the social insects.

Science, 149: 1064-1071.

Wilson, E.O. (1966)

Behaviour of social insects.

Symposium of the Royal Entomological Society of London, 3:
81-96.

Wilson, E.O. (1971)

The insect societies.

The Belknap Press of Harvard University Press, Cambridge,
Massachusetts. 548pp.

Wilson, E.O., Durlach, N.I. and Roth, L.M. (1958)

Chemical releasers of necrophoric behaviour in ants.

Psyche, Cambridge, 65 (4): 108-114.

+ Howick, C.D. (1970)

Eradication considerations.

Proceedings of the Termite Conference and Instruction course
for the Victorian Pest Control Industry, Australia. pp. 77-78.